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Weeds

JOURNAL OF THE WEED SOCIETY OF AMERICA

VOLUME 8 JULY 1960 NUMBER 3

Effect of 2,4–Dichlorophenoxyacetic Acid on the Nucleic Acid and Protein Content of Seedling Tissue[1]

S. H. WEST, J. B. HANSON and J. L. KEY[2]

ONE of the most marked effects of herbicidal concentrations of 2,4-D (2,4-dichlorophenoxyacetic acid) is the swelling and proliferation of basal stem tissues. Sell et al. (15) reported a large increase in the protein content of stems of bean plants treated with 2,4-D. Shaw, et al. (16) observed an increase of total protein in plants treated with 2,4-D and Rebstock, et al. (14) found the nucleic acid content to double in the stems of bean plants treated with 2,4-D. Rebstock et al. postulated that nucleic acid was involved in the unusual growth and development of the plant. Skoog (17) has presented an attractive hypothesis linking auxin action with nucleic acid metabolism. Current biochemical investigations leave little doubt that RNA (ribonucleic acid), particularly of the microsome fraction of the cytoplasm, is involved in protein synthesis (1). Furthermore, RNA appears to be implicated in oxidative phosphorylation (6) and ion absorption (8, 21), two processes known to be affected by 2,4-D (4, 12, 20).

The experiments reported here are the initial results of a study to determine if the herbicidal effects of 2,4-D are linked to aberrations in nucleotide metabolism. The results suggest that this may be true, but additional basic knowledge of the biochemistry of nucleotide metabolism in growing cells will be required if the question is to be fully answered.

METHODS AND MATERIALS

Greenhouse studies: Cucumber (Long Green) seed was lightly dusted with Spergon (U. S. Rubber Co.) and planted in potting soil, using 12-ounce waxed cups as containers. The plants were thinned to one

[1]Contributed from the Department of Agronomy, University of Illinois. This research was supported in part by Grant G-3358, National Research Foundation, and represents a portion of a thesis submitted by the senior author for the Doctor of Philosophy degree.

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per cup. When the second leaf appeared, randomly selected plants were sprayed with 5 ml of a commercial formulation of 2,4-D or 2,4,5-T (2,4,5–trichlorophenoxyacetic acid) amine. Concentrations of acid equivalent used are indicated in Figure 1 and Table 1. Treatments were made in quadruplicate. The soil surface was covered during spraying and care was taken to prevent drift.

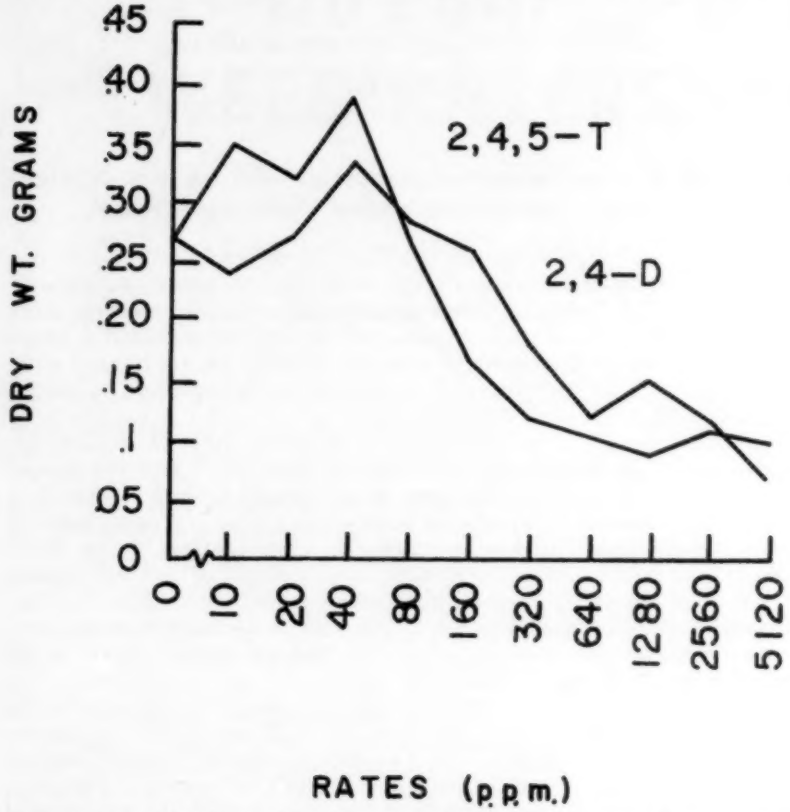

Figure 1. Dry weights of above ground part of cucumber plants 4 weeks after spraying at 2 leaf stage with 2,4-D and 2,4,5-T.

The cytoplasmic RNA and protein content of the leaves present at time of spraying and stem tissue from cotyledons to soil level (Table 1) were determined from tissue homogenates. The weighed fresh tissue was homogenized for 2 minutes in a glass homogenizer with a power driven teflon pestle, using 0.5 M sucrose with 0.067 M potassium phosphate (pH 6.8) and 0.001 M EDTA (ethylenediamine tetraacetic acid) as the medium. The debris, nuclei and some of the chloroplasts were removed by centrifuging at 1000 x g for 5 minutes at 0°C. Aliquots of the cleared homogenate were precipitated in cold 10% trichloroacetic acid. The precipitate was washed in

cold trichloroacetic acid, dissolved in 0.1 N NaOH and aliquots were analyzed for nitrogen by digestion and nesslerization. Protein was estimated as N x 6.25.

Other aliquots of the homogenate were precipitated with cold 0.2N $HClO_4$ and the precipitate was washed in the $HClO_4$ by suspension and resedimentation. The precipitate was extracted twice with 2:2:1 ethanol-ether-chloroform. The RNA was extracted from the defatted pellet by the hot perchloric acid method of Ogur and Rosen (13) for DNA (desoxyribonucleic acid). Ribonucleic acid content of the extracts was determined by 260–290 mμ absorption in a Beckman DU spectrophotometer referred to a standard curve with yeast RNA. The RNA analysis was verified by the orcinol method for ribose determination (23) and was shown free of detectable DNA by Stumpf's method (18). Duplicate analyses were made on four separate plants and the entire experiment was repeated.

Isolated tissue studies: Cucumber (Long Green) seed and corn (WF9 x M14) seed were germinated for three days in the dark at 28°C. Seeds were planted on paper toweling covering acid-washed gravel in pyrex baking dishes. Deionized water was added to the level of the paper. Seeds were lightly dusted with Spergon to prevent mold growth. Sections 1½ cm long of hypocotyl cut from just below the cotyledonary node or mesocotyl cut just below the coleoptilar node of 3-day old etiolated seedlings were weighed and allowed to grow overnight in the dark at 26°C in 10^{-3} M potassium phosphate (pH 6.5) and 2,4-D treatments as indicated in Table 2. Inhibitory concentrations of 500 or 800 ppm 2,4-D were used, and are summarized without distinction. In the experiments represented in Table 4, two successive 1 cm sections were cut from just below the coleoptile node of 3-day old etiolated corn seedlings. The tissue was then incubated for 11 hours on 10^{-3} M phosphate buffer, 1% sucrose and with indicated concentrations of 2,4-D.

After determining final fresh weights, the tissue was homogenized in 0.5 M sucrose, cleared of cell debris and nuclei, and assayed for RNA as described in the previous section. In later experiments (Table 3) duplicate 5 ml aliquots of the cleared homogenate (15 ml total volume) were sucessively centrifuged at 20,000 x g for 15 min. and 100,000 x g for 60 min. to sediment mitochondria and microsome fractions respectively. The mitochondria and microsomal pellets were suspended in water and aliquots were taken for protein analysis by the method of Lowery et al. (10), and for RNA extraction and analysis as above. Total protein and NRA analyses were made on aliquots of the cleared homogenate, again using the protein method of Lowery et al. and the described RNA extraction. Results of analyses are expressed per gram initial fresh weight.

RESULTS AND DISCUSSION

Preliminary greenhouse experiments were conducted to determine the growth response of cucumber plants to 2,4-D and 2,4,5-T. Figure 1 gives the results in terms of dry weight 4 weeks after application.

Both herbicides increased dry weight at low concentrations, but the increase was accompained by some epinasty and stem swelling. Proliferation of lower hypocotyl tissue was noted at 160 and 320 ppm. Concentrations of 2,4,5-T in excess of 1280 ppm and of 2,4-D in excess of 2560 ppm quickly resulted in leaf burn and death.

Under the conditions of these experiments, the minimum concentration of 2,4-D for a definite herbicidal effect was estimated to be 200 ppm; under field conditions, of course, plants weakened by lesser amounts might fail to survive. In the tissue studies which followed, 2,4-D concentrations between 400 and 800 ppm were assumed adequate to initiate physiological and biochemical responses underlying the death of both cucumber and corn plants.

Table 1 presents data on the RNA and protein content of stems and leaves 5 days after spraying with 400 ppm 2,4-D. The treatment

Table 1. Changes in fresh weight, protein nitrogen and ribonucleic acid of leaves and hypocotyl of cucumber plants following 2,4-D treatment.

Treatment	Tissue	Fresh wt gm/4 plants	Protein N mg/gm fr wt	RNA mg/gm fr wt
Control	Leaves	3.22	3.31	0.50
	Hypocotyl	1.94	0.39	0.08
2,4-D	Leaves	2.58	3.09	0.58
	Hypocotyl	2.22*	0.70*	0.40**

*Interaction significant, 5% level.
**Interaction significant, 1% level.

produced leaf epinasty and swelling and bending of the stems. The analyses confirm the observations of Rebstock et al. (14) and Sell et al. (15); leaf growth is restricted and stem growth is increased (although abnormally), with significant increases in RNA and protein content of the hypocotyl.

Table 2 summarizes experiments run to determine if isolated stem tissue would also respond to 2,4-D with increased levels of RNA.

Table 2. Changes in ribonucleic acid content of cucumber hypocotyl and corn mesocotyl sections floated on 2,4-D solutions.

2,4-D	Pct increase in fresh wt.	Mg RNA per gm initial fresh weight					
		Experiment					Average
		1	2	3	4	5	
Cucumber hypocotyl							
Control	17.6	.586	.590	.643	.613	.700	.626
5 ppm	32.6	.571	.570	.667	.589	.684	.616
500–800 ppm	20.0	.621	.655	.717	.730	.841	.713**
Corn mesocotyl							
Control	12.9	.497	.537	.729	—	—	.588
5 ppm	32.7	.459	.507	.655	—	—	.540*
500–800 ppm	13.5	.706	.737	.905	—	—	.783**

*Significant, 5% level.
**Significant, 1% level.

Corn mesocotyl was used as representative tissue from a species resistant to 2,4-D. Corn and cucumber tissue responded to 2,4-D in a similar manner; with a single exception, low concentrations of 2,4-D decreased and high concentrations increased the level of RNA. The exceptional result with 5 ppm 2,4-D (cucumber hypocotyl, Experiment 3 in Table 2) could represent an analytical error, since it was not found again, Day to day variations in control levels of RNA appeared to be correlated with small differences in the size of the seedlings; seedlings which were smaller than usual tended to have higher concentrations of RNA in the stem sections.

With the knowledge that 2,4-D affected RNA levels in isolated tissue as well as in the intact plant, somewhat more detailed analyses were carried out. Cytoplasmic protein was determined as well as RNA, and the microsomal and mitochondria fractions were analyzed for these constituents. Particular attention was given to the effects of herbicidal concentrations of 2,4-D, and analyses of the tissue prior to incubation were included. Typical results with cucumber hypocotyl and corn mesocotyl are given in Table 3.

Table 3. Changes in fresh weight and in the total and particulate RNA and protein of cucumber and corn sections incubated in 2,4-D.[a]

2,4-D ppm	% Increase in fresh weight	Mg RNA/gm fresh weight			Mg protein/gm fresh weight		
		Cyto-plasm	Mito-chondria	Micro-somes	Cyto-plasm	Mito-chondria	Micro-somes
		Cucumber hypocotyl					
Initial	—	.813	.267	.373	5.71	2.10	1.36
0	27.3	.447	.109	.237	3.49	1.14	0.88
200	30.7	.550	.094	.340	4.14	1.10	1.07
300	29.1	.577	.097	.368	4.47	1.11	1.13
800	10.2	.657	.100	.445	4.72	1.26	1.23
		Corn mesocotyl					
Initial	—	.939	.364	.423	6.72	2.69	1.63
0	23.1	.473	.323	.045	5.03	2.64	0.60
200	42.5	.547	.133	.150	5.48	2.19	1.25
300	36.1	.547	.133	.180	5.82	2.25	1.30
800	14.8	.746	.240	.240	6.34	2.70	1.36

[a]Sections excised and treated as in Table 2. Incubation was in the dark for 15 hours at 28°C. Tissue was homogenized, centrifuged and analyzed as described in Methods.

The most striking change in cytoplasmic RNA and protein was that which accompanied endogenous growth in 10^{-3} M phosphate buffer (no 2,4-D). About one half of the RNA (.813 to .447) and from 25–40% of the protein (.571 to .349) was lost in 15 hours. Such losses would not be expected to occur with cell expansion in stem tissue of the intact plant, where cell growth is accompanied by protein synthesis (19). Christiansen and Thimann (2) reported a considerable plasma protein synthesis during endogenous growth of pea epicotyl sections, but apparently subsequent work in the same laboratory has not verified this observation (22). In a large number of experiments with sections of cucumber, corn and soybean stem tissue, we have invariably found endogenous growth to lower RNA and

protein content, the absolute amount appearing to vary with species, maturity of tissue, time and temperature. However, no detailed investigation of this phenomenon has been made as yet.

For purposes of this investigation, it must be recognized that the growth of excised tissue is somewhat abnormal and herbicide effects noted with isolated tissue may not be fully applicable to the intact plant. However, the use of isolated tissue in auxin studies is firmly established, and since growth promotions and inhibitions are obtained, it can be assumed that the basic biochemical functions of the auxin-herbicides are operative.

As shown in Table 3, increasing concentrations of 2,4-D in the herbicidal range increased the total cytoplasmic RNA and protein relative to the buffer control. The question arises as to whether 2,4-D inhibited the metabolic degradation of RNA and protein (initial vs. no 2,4-D), or if these constituents were being resynthesized. Further investigation of this point led to the finding illustrated in Table 4; if relatively mature basal tissues are used, a net increase in RNA and protein over initial levels can be induced by 2,4-D thus indicating that synthesis is involved. It is not clear why a net increase can only be demonstrated in the more mature tissue, but the finding correlates with common observation that stem thickening and proliferation as a result of 2,4-D application are most marked in basal regions. It is probably also significant that the more mature tissue does not show a marked degradation of RNA and protein with endogenous growth or with low concentrations of 2,4-D (Table 4).

Table 4. Changes in the protein and ribonucleic acid content of corn mesocotyl sections incubated in 2,4-D.[a]

Section cm from coleoptile	2,4-D ppm	Pct increase in fresh weight	Mg protein gm fr wt	Mg. RNA gm fr wt
0.0–1.0	Initial	—	7.90	1.98
	0	21.5	6.30	1.36
	5	55.5	5.95	1.08
	800	18.5	7.25	1.89
1.0–2.0	Initial	—	3.60	0.34
	0	10.0	3.50	0.29
	5	17.5	3.75	0.31
	800	7.5	4.35	0.42

[a]Solutions remained clear with no perceptible bacterial contamination.

Analyses for relative changes in RNA and protein of the mitochondrial and microsomal fractions are difficult to interpret. The microsomal RNA of cucumber was often noted to be increased by high concentrations of 2,4-D to levels above those of the initial tissue (Table 3). Although this did not occur in comparable sections of corn mesocotyl, the proportionate decline in microsomal RNA with endogeneous growth was greater in corn, which may account for the difference. Relative to the buffer control, both corn and cucumber tissue exhibited large increases in microsomal RNA. Microsomal RNA is implicated in protein synthesis (1), and it is conceivable that increases in this cytoplasmic fraction are associated with the large

increases in protein content found in the stems of 2,4-D treated plants (15). It will be noted in Table 3 that relative to buffer control, 2,4-D induces proportionately larger increases in microsomal RNA than in microsomal protein. The action of 2,4-D may be more directly concerned with RNA synthesis than with protein synthesis.

The changes in mitochondrial RNA and protein are even less subject to explanation than those of the microsomes. It should be emphasized that the "mitochondria" isolated from sucrose homogenates consist not only of mitochondria, but also much membranous material with adhering microsomal granules (11). Much of the RNA of the mitochondrial fraction can be dispersed by homogenizing in the presence of EDTA, but the microsomal RNA is also dispersed, a circumstance we wished to avoid in these experiments. Because of the heterogeneity of the mitochondrial fraction, no attempt will be made to interpret the change noted in Table 3.

Taken as a whole, these experiments indicate that nucleotide metabolism is profoundly affected by 2,4-D. So, of course, are many other biochemical and physiological processes, but the central role played by mono- and polynucleotides in metabolism suggests how it is possible for 2,4-D to induce manifold physiological effects. Mononucleotides are known to be necessary to respiration and energy transfer (9), lipide synthesis (7), amino acid activation (3), sugar metabolism (5), etc. Ribonucleic acid is instrumental in protein synthesis, and apparently has some role in oxidative phosphorylation (6) and salt uptake (8, 21). It appears that basic studies of the metabolism of nucleotides and polynucleotides in growing plant cells may provide some clue as to the site of 2,4-D action.

SUMMARY

It has been verified that herbicidal concentrations of 2,4-dichlorophenoxyacetic acid increase the ribonucleic acid and protein content of stem tissue. The same effect can be obtained with isolated sections of cucumber hypocotyl and corn mesocotyl, particularly if basal sections are used. The microsomal fraction of the cytoplasm exhibits marked increases in RNA with herbicidal 2,4-D concentrations. Low concentrations of 2,4-D tend to promote the loss of RNA and protein which accompany endogenous growth.

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The Degradation of Isolated Microsomes from Cucumber and Corn Seedlings[1]

S. H. West and J. B. Hanson[2]

As reported in the previous paper (19), 2,4–D (2,4–dichlorophenoxyacetic acid) caused marked changes in the RNA (ribonucleic acid) content of seedling stem tissue, particularly in the microsome fraction. The ribonucleoprotein particles of the microsome fraction participate in protein synthesis (1) and any abnormality in their metabolism could conceivably result in biochemical and physiological disorders leading to the death of the plant.

In order to further study the effect of 2,4–D on RNA metabolism, it seemed desirable to first determine the effect of 2,4–D application on the isolated microsome fraction. Suggestions have been made that 2,4–D could inhibit growth through chelation of metal ions (8, 9, 10, 11). Microsomal particles in plants are held together by magnesium and/or calcium ions in a ribonucleic acid-protein complex and in the presence of such a chelating agent as EDTA (ethylenediaminetetraacetic acid), are dispersed into smaller units (12, 14, 15).

The initial experiments indicated that 2,4–D caused a dissociation of microsome particles, presumably through a chelation of magnesium and calcium, followed by a degradation of RNA by endogenous enzymes, and the data were so reported at the meeting of the Weed Society of America in Memphis, Tennessee, January, 1958 (18). However, continued experimentation showed the presumed 2,4–D effect was due to excess potassium ion used in neutralization of the 2,4–D stock solutions. The pertinent data are reported here to clarify the previous report of a 2,4–D effect on microsomal RNA degradation.

Materials and Methods

Corn (WF9 × M14) and garden cucumber (Long Green) seedlings were grown for 3 days as previously described (19). Microsome particles were prepared by grinding weighed cucumber hypocotyl or corn mesocotyl for 3 minutes in a chilled mortar and pestle with 0.5 M sucrose. Cell debris, mitochondria and nuclei were removed by centrifuging at 20,000 × g for 10 minutes at 0° C. The cleared homogenate was centrifuged again at 100,000 × g for 60 minutes to sediment the microsomes. The microsomes were suspended in 0.5 M sucrose and 0.001 M phosphate buffer at pH 6.8 (unless otherwise stated). Duplicate samples were incubated with additives and experimental conditions as noted in the tables and figures. Incubation was

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carried out in test tubes on a shaker at room temperature (23°–26° C). Total volume was 5 ml; 2 ml of which were the microsome suspension. At the end of the incubation period (1 hour unless otherwise noted), the suspension was precipitated by adding $HClO_4$ to 0.2 N, and the precipitate was washed in 0.2 N $HClO_4$ by suspension and resedimentation. The RNA content of the microsome precipitate was determined by defatting and extracting as previously described (19).

In some experiments the degradation of the RNA was verified by also determining the increase in acid-soluble nucleotides. This was done by determining the 260–290 mμ absorbancy of the acid soluble material and referring the readings to an adenosine monophosphate standard for estimation of nucleotide. When 2,4–D was present in the incubation medium, the absorbancy of the herbicide was corrected for by use of the proper blanks.

The 2,4–D used in these experiments was four times recrystallized from ethanol-water and was placed in solution by titration with KOH to pH 6.8. Stock solutions were kept in the refrigerator.

In the experiment reported in Table 7, cucumber microsomes were incubated on a shaker at room temperature with 0.01 M KCl, 500 ppm 2,4–D and the potassium salts of the indicated additive (pH 6.8).

RESULTS AND DISCUSSION

Table 1 shows the effect of incubating the microsome fraction in varying concentrations of KCl with and without 2,4–D. The addition of KCl resulted in degradation of the microsomal RNA such that it no longer precipitated in ice cold 0.2 N $HClO_4$, particularly in 0.1 M KCl. High concentrations of potassium ion are known to

Table 1. The effect of 2,4–D on the potassium-activated RNA degradation in isolated cucumber microsomes.[a]

Treatment	Mg RNA/tube			
	None	0.02 M KCl	0.05 M KCl	0.1 M KCl
Buffer control	.604	.593	.510	.409
500 ppm 2,4–D	—	.563	.442	.404

[a]Acid-precipitable RNA was determined at the end of the incubation.

adversely affect the stability of the microsomal ribonucleoprotein granules from yeast (5) and their ability to incorporate amino acids into protein (17). Since the work reported here was done, Ts'o (14) has reported pea microsomal granules to be dissociated into subunits by potassium ion, which are subsequently degraded by endogenous ribonuclease. Potassium nitrate and potassium sulfate were as effective as the potassium chloride indicating that potassium ion was responsible for initiating the degradation. Phosphate ion had a special metabolic effect to be discussed below.

Although not shown in Table 1, several experiments established that carefully neutralized 2,4–D had no significant effect on the

degradation of microsomal RNA. However, in the presence of potassium, 2,4–D somewhat accelerated the degradation, except at the highest KCl concentration used (Table 1). No explanation for this 2,4–D effect was found. The amount of potassium added with the 2,4–D (approximately 0.002 M) was not adequate to explain the increased degradation. If 2,4–D were to act as a chelating ion, it would be expected to induce degradation of the RNA in the absence of potassium.

Table 2 shows that the degradation of the microsomal RNA induced by KCl plus 2,4–D was enzymatic, in that low temperatures and boiling prevent degradation. Boiling in itself degraded some

Table 2. Loss of acid-insoluble RNA from cucumber hypocotyl microsomes during incubation.

Treatment	Mg RNA/tube		
	26°C	26°C (boiled)[a]	2°C
Control	.351	.202	.351
0.05 M KCl + 500 ppm 2,4–D	.255	.214	.352
	Mg soluble nucleotide/tube		
Control	.012	.128	.011
0.05 M KCl + 500 ppm 2,4–D	.091	.119	.008

[a]Where indicated, the microsomes were boiled for 3 minutes prior to incubation.

RNA, but there is no further degradation during incubation. The loss in RNA with incubation is largely accounted for by an increase in cold-acid-soluble nucleotides.

Although incubation in the cold prevents enzymatic degradation into acid soluble nucleotides, it does not prevent dissociation of the microsomal RNA into sub-units. As shown in Table 3, treatment of

Table 3. The dissociation of cucumber and corn microsomes by potassium ion and EDTA in ice temperatures.[a]

Tissue	Additive	Mg RNA sedimented by centrifugation	Mg RNA ppt'd by $HClO_4$
Cucumber	H_2O	.190	.224
Cucumber	0.05 M KCl + 500 ppm 2,4–D	.096	.216
Cucumber	0.0023 M EDTA	.054	.216
Corn	H_2O	.382	.546
Corn	0.05 M KCl + 500 ppm 2,4–D	.300	.537
Corn	0.0023 M EDTA	.142	.540

[a]The isolated microsomes were suspended in ice cold 0.5 M sucrose and incubated or centrifuged for 1 hour at 0 degrees centigrade with 0.001 M phosphate buffer (pH 6.8). RNA was determined on the acid-precipitated RNA or resedimented RNA.

microsomes with KCl + 2,4–D or with EDTA at 0 degrees centigrade so disperses the microsomal ribonucleoprotein granules that they cannot be resedimented at 100,000 × g for 1 hour, but they can still be precipitated in cold acid.

Ts'o et al. (14, 15) made ultracentrifuge studies of the dissociation of microsomal ribonucleoprotein granules from peas. The sub-units formed were reassociated in the presence of magnesium and calcium. Tables 4 and 5 illustrate the inhibition of degradation found with addition of divalent ions to cucumber microsomes. Magnesium, calcium and manganese were equally effective. Table 4, in addition, shows the effectiveness of EDTA in inducing degradation. The addition of EDTA with KCl + 2,4–D to the microsome preparation gave somewhat less degradation than did EDTA alone, but the effect was never very large.

Table 4. The effect of ethylenediaminetetraacetic acid (EDTA) on activation of microsomal RNA degradation.

Activator	Mg RNA/tube
None	340
0.0002 M EDTA	.241
0.002 M EDTA	.149
0.02 M EDTA	.150
0.02 M EDTA + 0.01 M $MgSO_4$	.284
0.05 M KCl + 500 ppm 2,4–D	.238
0.05 M KCl + 500 ppm 2,4–D + 0.02 M EDTA	.183

Table 5. Inhibition of cucumber microsomal RNA dissociation by divalent ions.[a]

Treatment	Mg RNA/tube			
	Divalent ion added			
	None	Mg^{++}	Mn^{++}	Ca^{++}
None	.274	.274	.274	.274
0.05 M KCl + 500 ppm 2,4–D	.194	.280	.282	.272

[a]Mg, Mn and Ca were added as the chlorides in 0.01 M final concentration.

The addition of potassium phosphate to microsomes appeared to affect an increased degradation of RNA over that expected to result from the potassium ion. Table 6 gives representative data with the

Table 6. Dependence of RNA degradation upon potassium and phosphate.

Treatment	Mg RNA/tube				
	Initial	PO_4 concentration			
		0	0.001 M	0.01	0.1 M
Undialyzed microsomes					
Control	.243	.240	.231	.223	—
0.05 M KCl + 500 ppm 2,4–D	.243	.154	.133	.105	—
Dialyzed microsomes[a]					
Control	.453	.382	—	—	—
0.05 M KCl + 500 ppm 2,4–D	.453	.321	.310	.263	.400

[a]Microsomes dialyzed against 0.01 M KCl for 2 days at 3–5 degrees centigrade.

usual undialyzed microsome preparation and with microsomes dialyzed to reduce endogenous phosphate. Dialysis against KCl resulted in considerable endogenous degradation, probably due to removal of some of the binding magnesium and calcium. The addition of phosphate with KCl + 2,4–D somewhat increased the degradation of RNA even at levels as low as 0.001 M. With dialyzed preparations the effect of high concentrations of phosphate (0.1 M) were investigated. These resulted in a definite reversal of degradation. Since degradation is enzymatic (Table 2), the high phosphate concentrations might somehow inhibit enzymatic activity.

Arsenate as well as phosphate would promote RNA degradation, and ADP (adenosine diphosphate) would reverse the effect of phosphate (Table 7). Figure 1 shows that ADP reversal could not be

Table 7. Effect of phosphate, arsenate, potassium and adenosine diphosphate on degradation of microsomal RNA.

Additives	Mg RNA/tube		
	Initial	After 1 hr.	After 2 hrs.
0.01 M arsenate	.321	.192	.132
0.01 M phosphate	.321	.198	.132
0.01 M phosphate + 0.002 M ADP	.321	.313	.298

obtained with AMP (adenosine monophosphate). Collectively, these data suggest that the enzymatic degradation was due not only to ribonuclease, which hydrolytically cleaves the phosphate linkages of polyribonucleotides, but also to polynucleotide phosphorylase, which cleaves the linkages with phosphate. Polynucleotide phosphorylase, which has been found in plant material (4, 7), can make polyadenylate from ADP, and the formation of this compound could account for the apparent reversal of degradation as noted. Also the addition of ADP could shift the reaction equilibrium so that degradation could not proceed. The ineffectiveness of AMP would be expected as it could not be polymerized without first being phosphorylated. The mitochondria necessary for the formation of high energy phosphate had, of course, been removed in the initial centrifugation.

In general, this investigation failed to disclose a significant role for 2,4–D in the degradative metabolism of the ribonucleoprotein particles, although a small promotive effect of the herbicide in combination with 0.05 M KCl was found. This promotive effect of 2,4–D has not yet been explored, but it appears unlikely that its action is related to a chelating property of the molecule. A true chelate ion such as EDTA initiates degradation of RNA at very low concentrations (Table 4). It is possible that 2,4–D might in some way accelerate the subsequent metabolism of nucleotides released by ribonuclease or polynucleotide phosphorylase, thus shifting the reaction equilibrium of degradation. However, any reasonable speculation in this area will have to await direct experimentation.

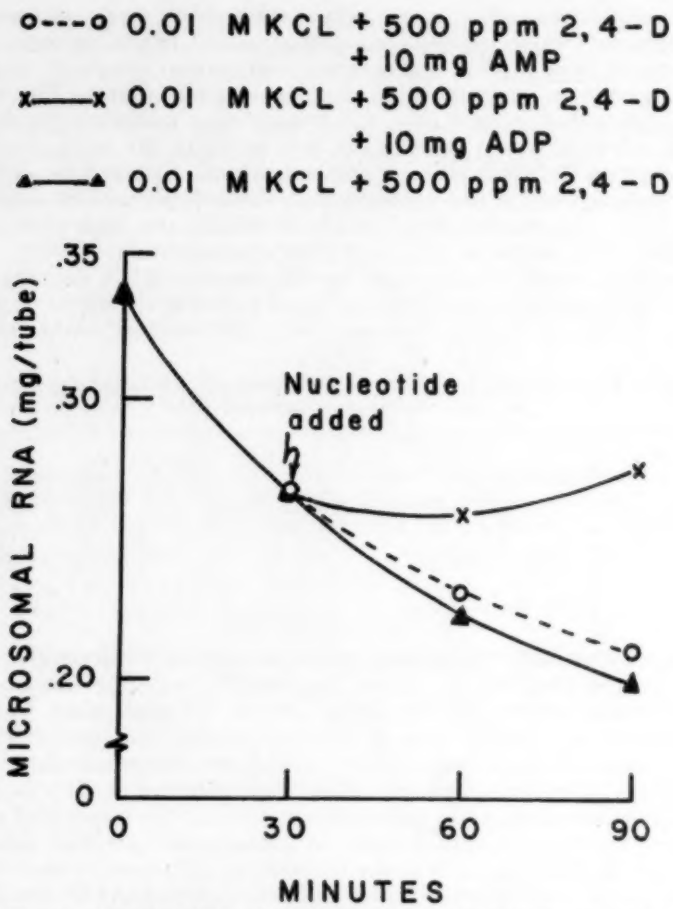

Figure 1. Degradation of isolated microsomal granules at room temperature. Replicated tubes of cucumber microsomes with 0.001 M phosphate buffer and 0.0005 M KCl + 500 ppm 2,4–D were incubated on a shaker. After 30 minutes ADP and ATP were added to tubes of two replicates. The RNA content of the microsomes was determined at the indicated intervals.

The experiments show certain facets of RNA metabolism which may be of use in the study of the action of 2,4–D, which as previously described affects RNA synthesis in high concentrations (19). A clear 2-phase process in the degradation of the microsome ribonucleoprotein granules was observed. First, the binding magnesium or calcium (the predominant divalent ions of the cell) were removed by potassium substitution or by chelation, producing sub-units which would not sediment at 100,000 × g. Second, these sub-units contained RNA which was susceptible to degradation by endogenous enzymes, presumably ribonuclease and polynucleotide phosphorylase. It is not

clear whether the RNA was subject to degradation because the divalent ions were removed or because sub-units were formed. The phosphate in RNA possesses one residual negative charge, and it is conceivable that binding of calcium or magnesium to adjacent phosphate groups might hinder enzymatic cleavage of the phosphate linkage.

Although potassium ion has long been known to stimulate auxin-induced growth (6, 13), the functional relationship has remained obscure. However, it is known that expansive growth in plants involves protein synthesis (2, 3), and since microsomal RNA is implicated in protein synthesis (1), it can be speculated that expansive growth occurs with a simultaneous microsomal RNA degradation. Lund et al. (12) and Ts'o and Sato (16) have described a transference of microsomal RNA to other cell fractions with cell growth, presumably auxin-induced. Small polynucleotide units may be needed for protein synthesis and other metabolic processes underlying growth; these could be obtained from the degradation of the microsome granules which the daughter cell inherits at cell division. The particles could be degraded during normal expansive growth by the substitution of monovalent ions such as potassium for the binding divalent ions, thus permitting the granules to disperse into sub-units which are then available for further metabolism. In this way, potassium ion may stimulate auxin-induced growth by making available the polynucleotides and mononucleotides needed in protein synthesis and other growth processes.

SUMMARY

It has been shown that isolated microsomal particles dissociate into sub-units upon incubation with KCl or EDTA and that 2,4–D has little effect on this process. These sub-units are further degraded into acid-soluble nucleotides by endogenous enzymes. A theory that potassium ion stimulates auxin-induced growth by accelerating the degradation of microsomal particles is discussed.

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Control of Weeds in Legume Seedings with 4–(2,4–DB), Dalapon, and TCA[1]

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THE small size and slow growth of legume seedlings make them susceptible to damage or elimination by weed competition. Various methods of establishment such as sowing in late summer, sowing with a small grain companion crop, or mowing have not been entirely successful. The increased use of weed-controlling chemicals has awakened considerable interest in the establishment of legumes with the aid of herbicides. Research workers are in agreement that 4–(2,4–DB) [4–(2,4–dichlorophenoxy)butyric acid] can be safely used to control broad-leaved weeds in legumes (2, 5, 6, 7, 9, 13, 14). However, there is some difference of opinion as to the rates of various formulations that can be safely used (2, 9, 13).

Weed grasses are not controlled by 4–(2–DB) and often the removal of the broad-leaved canopy encourages the growth of weed grasses. Kerr[3] showed that weed grasses are more injurious to birdsfoot trefoil than broad-leaved weeds. A number of workers have found that weed grasses can be controlled without injury to alfalfa and birdsfoot trefoil by rates of TCA (trichloroacetic acid) ranging from 4 to 9 lb/A and by rates of dalapon (2,2–dichloropropionic acid) ranging from 2 to 6 lb/A (1, 3, 4, 8, 10, 11, 12, 17). However, some workers (15, 16) report moderate to severe injury from both TCA and dalapon.

To obtain control of both broad-leaved weeds and weed grasses, combinations of herbicides appear to be necessary. Several workers report good results from using mixtures of 4–(2,4–DB) and dalapon to control both types of weeds (9, 15).

The first part of the work reported in this paper was designed to compare an amine with an ester of 4–(2,4–DB) at several rates to determine which rates of each herbicide give practical weed control with minimum injury to seedling alfalfa, red clover, and birdsfoot trefoil. Dalapon was also included as a mixture with 1 lb/A of an ester and of an amine. The second part of the work reported in this paper was designed to compare the effect of an ester formulation of 4–(2,4–DB) at 1 and 2 lb/A in combinations with dalapon at 2 and 3 lb/A and TCA at 8 lb/A.

METHODS AND MATERIALS

Ranger alfalfa, Kenland red clover, and European birdsfoot trefoil were planted on April 15, 1957, and on April 8, 1958 at rates of 12,

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[3]Kerr, H. D., Weed control studies in seedling birdsfoot trefoil (*Lotus corniculatus* L.). M.S. Thesis. University of Missouri, 1957.

12, and 10 lb/A, respectively. Seeding was done with a corrugated-roller type of seeder on a well-prepared seedbed of Mexico silt loam. Each legume was sown in a separate block and separate experiments were laid out in each block. Plots were 7 feet wide and 20 feet long.

Excellent germination and emergence of all legumes occurred during both years. Heavy infestations of smartweeds (*Polygonum* spp.) and pigweeds (*Amaranthus* spp.) were present early in the season. Few grasses were present early in the season but plants of crabgrass (*Digitaria* spp.) and barnyardgrass (*Echinochloa crusgalli*) were numerous in mid-summer.

The legumes were harvested in the bloom stage by mowing a swath 3 ft wide and 20 ft long through the center of each plot. The green forage from each strip was weighed. A representative sample of forage was dried in an oven to determine the percent dry weight. Another representative sample from each plot of the first harvest of each year was hand-separated to determine the percentages of oven-dry weight of weeds and legumes. In the second cutting, weed infestation was generally light and the percentages of weeds and legumes were estimated visually. The percentages were applied to the total oven-dry weight to determine the yields per acre of each component.

Plant counts were made in late summer within four 6- by 36-inch quadrats placed at random in each plot to determine the effects of the treatments on the stand of the legumes.

Rainfall during the summer of 1957 was below normal and legumes suffered from lack of moisture during July. In 1958, however, rainfall was above normal and the growth of legumes and weeds was abundant.

Comparison of an amine and ester of 4–(2,4–DB). Post-emergence treatments of a butoxyethanol ester and a triethanolamine salt of 4–(2,4–DB) were applied to alfalfa and birdsfoot trefoil at rates of ½, 1, and 2 lb/A. Red clover received treatments of ½, ¾, 1, 1½, and 2 lb/A of each 4–(2,4–DB) formulation. In addition, each legume received treatments of 1 lb/A of each formulation mixed with 2 lb/A of dalapon. (Rates of 4–(2,4–DB) and dalapon refer to acid equivalent.) The experiments were designed as randomized complete blocks.

Spraying was done on May 27, 1957 and on May 27 and 28, 1958, when legumes and weeds were at the following heights: Alfalfa 6 to 10 in, birdsfoot trefoil 3 to 4 in, red clover 4 to 5 in, smartweeds 4 to 10 in, and pigweeds 4 to 6 in. All herbicides were applied in water at 40 gal/A and at 40 psi.

4–(2,4–DB) in combination with TCA or dalapon. The experiments were laid out as split-plot designs with these main treatments: 1. TCA at 8 lb/A; 2. dalapon at 2 lb/A; 3. dalapon at 3 lb/A; and 4. an untreated check. Sub-treatments consisted of an untreated check and treatments with 1 and 2 lb/A of mixed esters of 4–(2,4–DB). Each rate of 4–(2,4–DB) appeared twice in each main plot, once with supplemental treatments of 2 lb/A of dalapon applied later in the season, and once with no supplemental treatment. Main treatments with dalapon and TCA and sub-treatments with 4–(2,4–DB)

were applied to alfalfa on June 7, 1957, and on May 26, 1958; and to birdsfoot trefoil on June 7, 1957, and May 28, 1958. Where 4–(2,4–DB) was used as a sub-treatment, the material was applied alone or as mixtures with TCA or dalapon where these appeared as main treatments. At the time of treatment in 1957, alfalfa was 10, birdsfoot trefoil 4 to 5, smartweeds 12, pigweeds 8, and crabgrass 3 to 4 inches tall. In 1958, treatments were applied when alfalfa was 6 to 10, birdsfoot trefoil 3 to 4, smartweeds 6 to 8, pigweeds 6 to 8, and crabgrass 3 to 4 inches. Supplemental treatments with 2 lb/A of dalapon were applied after forage was cut at the hay stage.

RESULTS

Comparison of an amine and ester of 4–(2,4–DB).

Alfalfa. The use of yield data for the evaluation of injury to legumes is not always entirely sound because the data is often confounded by the weed growth in the experiment. All rates of herbicides in 1957 and all rates above ½ lb/A of the ester or 1 lb/A of the amine in 1958 decreased the yields of broad-leaved weeds in the first cutting to such an extent that weeds had little influence on the yields of alfalfa (Tables 1 and 2). In 1958, broad-leaved weed control by the ½ lb/A

Table 1. Yields of oven-dry seedling alfalfa treated with 4–(2,4–DB) and dalapon.

Rate lb/A		First cutting[a] lb/A			Second cutting[a] lb/A			Av. yield per year lb/A
4–(2,4–DB)	Dalapon	1957	1958	Av.	1957	1958	Av.	
Amine								
½		1,610	860	1,240	1,100	2,380	1,740	2,980
1		1,960	1,700	1,830	1,240	2,440	1,840	3,670
2		1,660	1,550	1,600	1,190	1,620	1,410	3,010
1	2	1,200	1,120	1,160	1,040	2,590	1,820	2,980
Ester								
½		1,590	1,510	1,550	1,130	2,240	1,690	3,240
1		1,490	1,540	1,520	1,540	2,030	1,790	3,300
2		1,220	1,280	1,250	1,070	1,470	1,270	2,520
1	2	1,220	1,330	1,270	1,230	2,560	1,890	3,170
None		1,460	440	950	1,060	2,530	1,800	2,750
LSD 5%		300	240	—	n.s.	440	—	—
1%		410	320	—	n.s.	590	—	—

[a]Forage was cut on June 20 and August 22, 1957, and on July 1 and August 14, 1958.

rate of both formulations and the 1 lb/A rate of the amine was appreciably less than by the other treatments. During both years 4–(2,4–DB) applied before the first cutting reduced yields of broad-leaved weeds appreciably in the second cutting. The broad-leaved weeds present in the second cutting were nearly all regrowth from the original stand. Yields of weed grasses tended to increase when competition from broad-leaved weeds was reduced (Tables 2 and 3). The increase in weed grasses was not large enough to affect appreciably the yields of alfalfa in the first cutting, but yields of these weeds were high in the second cutting.

Table 2. Yields of oven-dry broad-leaved weeds in seedling alfalfa treated with 4–(2,4–DB) and dalapon.

Rate lb/A		First cutting[a] lb/A			Second cutting[a] lb/A			Av. yield per year lb/A
4–(2,4–DB)	Dalapon	1957	1958	Av.	1957	1958	Av.	
Amine								
½		240	1,860	1,050	400	50	230	1,280
1		110	730	420	110	10	60	480
2		20	200	110	70	20	50	160
1	2	90	330	200	210	20	120	320
Ester								
½		160	790	480	130	10	70	550
1		120	240	180	130	20	70	250
2		20	210	120	90	10	50	170
1	2	60	310	190	90	20	50	240
None		1,000	3,160	2,080	770	440	610	2,690
LSD 5%		240	490	—	ns	ns	—	—
1%		330	620	—	ns	ns	—	—

[a]Forage was cut on June 20 and August 22, 1957, and on July 1 and August 14, 1958.

In 1957, dalapon applied in May had little effect on the growth of weed grasses in the second cutting, but in 1958 dalapon substantially reduced the amount of grasses (Table 3). Possibly the heavy rainfall in 1958 caused movement of dalapon to the root zone of the grass while this did not occur in 1957, when rainfall was low.

Table 3. Yields of oven-dry weed grasses in seedling alfalfa treated with 4–(2,4–DB) and dalapon.

Rate lb/A		First cutting[a] lb/A			Second cutting[a] lb/A			Av. yield per year lb/A
4–(2,4–DB)	Dalapon	1957	1958	Av.	1957	1958	Av.	
Amine								
½		290	50	170	1,550	970	1,260	1,430
1		160	60	110	1,400	1,620	1,510	1,620
2		330	130	230	2,080	2,260	2,170	2,400
1	2	20	10	20	1,650	660	1,150	1,170
Ester								
½		410	40	230	1,580	1,730	1,660	1,880
1		280	90	190	1,830	2,040	1,930	2,120
2		370	70	220	2,310	2,830	2,570	2,790
1	2	20	0	10	1,600	550	1,080	1,090
None		210	30	120	1,420	300	860	980
LSD 5%		130	ns	—	ns	850	—	—
1%		180	ns	—	ns	1,150	—	—

[a]Forage was cut on June 20 and August 22, 1957, and July 1 and August 14, 1958.

The highest yields of alfalfa were obtained during both years when 1 lb of the amine of 4–(2,4–DB) was used (Table 1). The comparatively lower yields of alfalfa from plots treated with 1 lb or more of the ester or 2 lb of the amine are probably due to injury by the herbicides although yields of weed grasses were somewhat higher in these plots and competition may have accounted for some of the reduction in alfalfa yield. Low yields of alfalfa with ½ pound of the amine were evidently due to poor control of broad-leaved weeds.

Control of these weeds was good with ½ lb of ester, but the yield of alfalfa was lower than the yield obtained with 1 lb of amine. The ester appeared to injure the alfalfa more severely at all rates than the amine as indicated by the differences in yields.

The use of dalapon in addition to 1 lb of 4–(2,4–DB) reduced the yields of alfalfa as compared to 1 lb of 4–(2,4–DB) alone. The average reductions per year were 130 lb/A where the ester was used and 700 lb/A where the amine was used.

In the first cutting in 1957, the two better herbicide treatments increased yields of alfalfa over the check by 200 and 500 lb/A. These increases are not large but the appreciable reduction in the yield of weeds and the improvement in quality of hay may be justification for treatment. In the first cutting in 1958, the 1- and 2-lb rates of amine and the ½ and 1-lb rates of ester increased yields of alfalfa by more than 1000 lb/A as compared with the check.

Birdsfoot trefoil. The untreated plots of birdsfoot trefoil were clipped for weed control in 1957, so yields of the legume or weeds were not measured. In 1958 the plots were not clipped for weed control, so yields were taken (Table 4).

Table 4. Yields of oven-dry seedling birdsfoot trefoil and weeds treated with 4–(2,4–DB) and dalapon.

Rate, lb/A		Trefoil[a] lb/A			Brd-lvd weeds lb/A			Weed grasses lb/A		
4–(2,4–DB)	Dalapon	1957	1958	Av.	1957	1958	Av.	1957	1958	Av.
Amine										
½		340	170	260	1,100	3,000	2,050	1,890	660	1,280
1		220	130	180	1,810	2,240	2,020	1,140	1,080	1,110
2		180	310	240	1,280	1,330	1,300	1,860	920	1,390
1	2	530	1,000	760	1,250	1,070	1,160	350	100	230
Ester										
½		270	320	300	970	1,470	1,220	1,620	1,480	1,550
1		170	310	240	940	1,180	1,060	2,020	1,660	1,840
2		60	240	150	310	740	530	2,470	1,790	2,130
1	2	570	960	770	960	1,390	1,180	600	160	380
None		—	20	—	—	7,000	—	—	140	—
LSD 5%		270	330	—	750	1,050	—	910	740	—
1%		360	450	—	1,020	1,430	—	1,240	1,010	—

[a]Forage was cut on July 19, 1957, and on July 23, 1958.

Previous experiments show that birdsfoot trefoil is especially sensitive to competition from grasses that come in after broad-leaved weeds are removed. The slow-growing trefoil is not competitive enough to keep down the weed grasses, and consequently the best yields of trefoil in this experiment occurred where 2 lb/A of dalapon was used to control weed grasses along with 1 lb/A of 4–(2,4–DB) for broad-leaved weed control.

Birdsfoot trefoil was harvested later than the alfalfa and the weeds had an opportunity to recover to some extent. For this reason treatments with 4–(2,4–DB) do not appear as effective in reducing broad-leaved weed yields in trefoil as they do in alfalfa (Tables 1 and 4). Also, the trefoil was not competitive enough to appreciably reduce

the growth of broad-leaved weeds. All treatments with 4–(2,4–DB), however, reduced the yields of broad-leaved weeds considerably as shown by the 7,000-pound yield of weeds from the check plot in 1958 compared with the yields of the treated plots (Table 4). Weed yields were still so large in the treated plots that it was nearly impossible to distinguish between injury from the herbicides and injury from weeds.

At the same rates there were no significant differences in yields of birdsfoot trefoil between the amine and ester. There was a tendency for the plots treated with ester to yield less trefoil than plots treated with amine in 1957. In 1958 the situation was reversed and higher yields of trefoil resulted from use of the ester. During both years the ester tended to control broad-leaved weeds better than the amine.

Red clover. Very little increased control of broad-leaved weeds was obtained with rates higher than ¾ lb/A of either the amine or the ester formulation of 4–(2,4–DB) in the first cutting of red clover (Table 6). Generally better broad-leaved weed control was obtained with the ester than with the amine, especially at the ½ lb/A rate. Virtually no broad-leaved weeds were found in the second cutting of red clover. Weed grasses were numerous in the first cutting, but the legume recovered so rapidly after cutting that weeds were crowded out in the second cutting (Table 7).

Table 5. Yields of oven-dry seedling red clover treated with 4–(2,4–DB) and dalapon.

Rate lb/A		First cutting[a] lb/A			Second cutting[a] lb/A			Av. yield per year lb/A
4–(2,4–DB)	Dalapon	1957	1958	Av.	1957	1958	Av.	
Amine								
¼		1,450	1,540	1,500	1,200	2,050	1,620	3,120
½		1,820	1,960	1,890	1,430	2,030	1,730	3,620
¾		1,970	2,070	2,020	940	1,930	1,440	3,460
1		1,830	1,770	1,800	1,380	1,860	1,620	3,420
1¼		2,010	1,830	1,920	1,810	2,130	1,970	3,890
1½		1,510	1,970	1,740	1,530	1,960	1,750	3,490
2		170	1,090	630	830	1,640	1,230	1,870
1	2							
Ester								
¼		1,780	2,260	2,020	940	1,860	1,400	3,420
½		2,120	1,870	2,000	2,020	1,940	1,980	3,980
¾		1,840	2,110	1,980	1,280	1,710	1,490	3,470
1		2,120	2,320	2,220	1,640	2,270	1,950	4,170
1¼		1,850	2,090	1,970	1,050	2,020	1,530	3,500
1½		2,020	2,040	2,030	1,330	2,170	1,750	3,780
2		930	1,120	1,030	830	1,800	1,320	2,340
1	2							
None		900	1,340	1,120	220	1,800	1,010	2,130
LSD 5%		720	ns	—	730	ns	—	—
1%		980	ns	—	980	ns	—	—

[a]Forage was cut on July 10 and August 29, 1957, and on July 19 and September 13, 1958.

Dalapon nearly eliminated the weed grasses in red clover but it injured the legume to such an extent that yields were seriously reduced (Table 5). Red clover apparently is more tolerant to 4–(2,4–DB) than alfalfa and birdsfoot trefoil because no reduction in

Table 6. Yields of oven-dry broad-leaved weeds in seedling red clover treated with 4–(2,4–DB) and dalapon.

Rate lb/A		First cutting[a] lb/A			Second cutting[a] lb/A			Av. yield per year lb/A
4–(2,4–DB)	Dalapon	1957	1958	Av.	1957	1958	Av.	
Amine								
½		1,360	1,980	1,670	0	120	80	1,750
¾		310	810	560	0	20	10	570
1		220	870	540	260	50	160	700
1¼		190	960	570	0	60	30	600
1½		170	750	460	0	10	0	460
2		0	970	490	0	0	0	490
1	2	680	1,000	840	220	220	220	1,050
Ester								
½		580	450	510	0	30	10	530
¾		110	360	240	0	0	0	240
1		190	380	280	0	40	20	300
1¼		50	360	210	0	30	20	220
1½		0	70	40	0	40	20	50
2		60	120	110	0	20	10	120
1	2	370	870	620	250	90	170	790
None		2,650	2,650	2,650	60	120	90	2,740
LSD 5%		770	970	—	—	—	—	—
1%		1,040	1,300	—	—	—	—	—

[a]Forage was cut on July 10 and August 29, 1957, and on July 19 and September 13, 1958.

Table 7. Yields of oven-dry weed grass in seedling red clover treated with 4–(2,4–DB) and dalapon.

Rate lb/A		First cutting[a] lb/A			Second cutting[a] lb/A			Av. yield per year lb/A
4–(2,4–DB)	Dalapon	1957	1958	Av.	1957	1958	Av.	
Amine								
½		550	280	410	0	90	50	460
¾		850	870	860	0	60	30	890
1		630	640	630	0	20	10	640
1¼		860	520	690	0	150	70	770
1½		700	720	710	0	110	60	770
2		390	690	540	0	60	30	570
1	2	0	50	30	0	30	20	40
Ester								
½		910	770	840	0	80	40	880
¾		740	1,020	880	0	70	40	920
1		670	460	560	0	60	30	590
1¼		650	570	610	0	20	10	620
1½		1,190	800	990	0	70	30	1,030
2		730	720	720	0	190	100	820
1	2	0	10	10	0	0	0	10
None		350	420	390	0	10	0	390
LSD 5%		520	ns	—	—	—	—	—
1%		700	ns	—	—	—	—	—

[a]Forage was cut on July 10 and August 29, 1957, and on July 19 and September 13, 1958.

yield occurred even at the 2 lb/A rate (Tables 1, 4 and 5). Where the ½-lb rate of the amine was used, yields of the first cutting of red clover were somewhat low because of poor broad-leaved weed control.

Density of legume stands. The number of alfalfa plants present in the stand was unaffected by treatments (Table 8). In birdsfoot trefoil,

Table 8. Effect of 4–(2,4–DB) and dalapon on the stand of alfalfa, birdsfoot trefoil, and red clover indicated by counts of plants in six square feet of plot.

Rate, lb/A		Alfalfa			Birdsfoot trefoil			Red clover		
4–(2,4–DB)	Dalapon	1957	1958	Av.	1957	1958	Av.	1957	1958	Av.
Amine										
½		89	66	78	60	48	54	92	64	78
¾		—	—	—	—	—	—	106	67	87
1		85	65	75	58	26	42	106	72	89
1½		—	—	—	—	—	—	102	78	90
1½		—	—	—	—	—	—	110	71	91
2		80	70	75	52	42	47	99	62	81
1	2	84	66	75	79	55	67	51	62	57
Ester										
½		85	60	73	57	38	48	92	71	82
¾		—	—	—	—	—	—	107	72	90
1		90	65	78	52	21	37	106	72	90
1½		—	—	—	—	—	—	104	67	86
1½		—	—	—	—	—	—	97	75	86
2		78	61	70	34	29	32	89	72	81
1	2	87	72	80	82	45	64	64	64	64
None		89	71	80	69	18	44	79	79	75
LSD 5%		ns	ns	—	13	21	—	19	ns	—
1%		ns	ns	—	18	28	—	26	ns	—

[a]Counts of legume plants were made in four 6- by 36-inch quadrats in each plot on the following dates:

	1957	1958
Alfalfa	August 31	August 21
Red clover	August 2	September 9
Trefoil	August 2	August 12

however, the number of plants was increased about 50 percent by the use of a combination of 1 lb/A of either formulation of 4–(2,4–DB) and 2 lb/A of dalapon. The reduced competition from weed grasses was important in increasing the birdsfoot trefoil population. There is an indication that the birdsfoot trefoil population was reduced when 2 lb/A of ester was used. Some reduction in birdsfoot trefoil yield also occurred at this rate compared with the 1 lb/A rate of ester.

The use of dalapon on red clover in 1957 reduced the stand of this legume significantly as compared with other treatments. No significant reduction occurred in 1958. The population of red clover was not reduced by rates or formulations of 4–(2,4–DB).

4–(2,4–DB) in combination with TCA or dalapon.

Alfalfa. Post-emergence applications of TCA and dalapon, as main treatments, cause yellowing of the seedling alfalfa, but the discoloration disappeared after about 2 weeks. Yellowing was most severe on plots treated with TCA. Dalapon or TCA mixed with 4–(2,4–DB) caused more discoloration than either chemical alone. This injury was severe enough to reduce yields of alfalfa in the first cutting (Table 9). Plots receiving main treatments of TCA and dalapon yielded less alfalfa than plots that were not treated even though the amounts of weed grasses were reduced by over 500 lb/A on treated plots as compared with non-treated. These differences in yields of alfalfa were significant in 1957 but not in 1958. During

Table 9. Oven-dry yields of alfalfa and weeds in a new alfalfa seeding as affected by herbicides in 1957 and 1958 (first cutting).[a]

Main treatments[b] lb/A	Sub-treatments lb/A 4–(2,4–DB)	Oven-dry yields lb/A								
		Alfalfa			Broad-leaved weeds			Weed grass		
		1957	1958	Av.	1957	1958	Av.	1957	1958	Av.
TCA 8	1	720	2,030	1,370	83	150	120	97	48	73
	2	650	1,770	1,210	100	24	62	130	130	130
	None[c]	—	420	420	—	3,660	3,660	—	10	10
	Av.	690	1,410	1,360	92	1,280	800	120	65	85
Dalapon 2	1	730	1,780	1,260	350	100	230	87	14	51
	2	790	2,360	1,580	120	43	81	85	5	45
	None[c]	—	1,150	1,150	—	4,050	4,050	—	0	0
	Av.	760	1,760	1,360	240	1,400	400	86	6	38
Dalapon 3	1	740	2,040	1,390	240	150	200	77	20	49
	2	690	2,130	1,410	130	65	96	70	23	47
	None[c]	—	750	750	—	3,350	3,350	—	9	9
	Av.	720	1,640	1,270	190	1,190	790	74	17	40
None	1	1,070	2,360	1,720	110	8	59	470	900	680
	2	1,080	2,420	1,750	58	29	44	520	280	400
	None[c]	—	460	460	—	3,700	3,700	—	860	860
	Av.	1,070	1,750	1,480	84	1,250	780	500	680	610
Sub-treatment averages	1	820	2,050	1,430	200	100	150	180	250	210
	2	800	2,170	1,490	100	40	70	200	110	160
	None[c]	—	690	—	—	3,690	—	—	220	—
LSD 5%										
Main treatments		240	ns		ns	—[d]		63	—[d]	
Sub-treatments		ns	311		83	—		ns	—	

[a]Forage was cut on July 2, 1957, and on July 8, 1958.
[b]Main treatments with TCA and dalapon were applied alone or as mixtures with 4–(2,4–DB) of the sub-treatments on June 7, 1957, and May 26, 1958.
[c]These sub-treatments were mowed early for broad-leaved weed control in 1957.
[d]Analysis of variance was not calculated because of the nature of the data.

both years, there was a tendency for plots treated with 8 lb/A of TCA to yield less alfalfa than plots treated with dalapon, although the differences were not statistically significant. Plots treated with 2 lb/A of dalapon yielded slightly more alfalfa than plots treated with 3 lb, indicating that injury was more severe with 3 lb than with 2 lb.

During both years, yields of alfalfa from plots treated with 1 or 2 lb/A of 4–(2,4–DB) were not greatly different. The non-treated plots within each main treatment were mowed early for broad-leaved weed control in 1957 and yields of forage from the first cutting were not measured; however, in 1958 the alfalfa was all mowed at the same time and yields for all plots are given. The data indicate that treatments with 4–(2,4–DB) significantly reduced the yields of broad-leaved weeds and increased yields of alfalfa considerably as compared with no treatment.

In 1957, the 2 lb rate of 4–(2,4–DB) controlled broad-leaved weeds significantly better than the 1 lb rate, although broad-leaved weeds were controlled adequately at both rates. Little difference in the effect of the two rates on broad-leaved weed control was apparent in 1958. TCA and dalapon had little effect on broad-leaved weeds.

Appreciable reduction in yields of weed grasses was obtained during both years by use of dalapon and TCA as compared with the

check. Dalapon nearly eliminated all weed grasses and appeared to be slightly superior to TCA.

Yields of alfalfa from the second cuttings were not appreciably affected by main treatments with dalapon or TCA (Table 10). Sub-treatments with 4–(2,4–DB) at 1 or 2 lb/A in 1957 significantly increased yields of alfalfa over the non-treated plots and also over plots treated with 4–(2,4–DB) plus 2 lb/A of dalapon. The use of 4–(2,4–DB) evidently caused an increase in alfalfa yields because of better broad-leaved weed control during a season when moisture was limited. Where 4–(2,4–DB) was used with supplemental treatments of dalapon, yields may have been depressed by the dalapon. The differences in yields, however, were low and the L.S.D. at the 5% level was only 122 lb/A. Differences between yields because of treatments were not significant in 1958. Rainfall in 1958 was adequate and moisture used by weeds had little apparent effect on yields of alfalfa.

Broad-leaved weeds were generally sparse in the second cutting during both years, but residual effects of 4–(2,4–DB) reduced the yields of broad-leaved weeds in plots treated with this herbicide (Table 11).

The effectiveness of the main treatments with dalapon and TCA had diminished at the time of the second cuttings, but the reduced stands of weed grasses from initial treatment resulted in reduced yields of these grasses at the time of the second cutting in 1957 and 1958 (Table 12).

Supplemental treatments with 2 lb/A of dalapon after the first cutting nearly eliminated all weed grasses. In 1958, yields of weed grasses were greater in plots receiving 4–(2,4–DB) than in the check plots. The reduction of broad-leaved weeds in the treated plots permitted weed grasses to grow because of reduction in competition.

Abundant rainfall during 1958 promoted good growth of seedling alfalfa and allowed a third cutting to be taken in September. Supplemental treatments with dalapon following the first cutting reduced yields of alfalfa significantly in the third cutting as compared to other treatments (Table 13).

None of the herbicide treatments affected the number of alfalfa plants in the stand (Table 14).

Birdsfoot trefoil. Because birdsfoot trefoil grew slower during the year of seeding than alfalfa it was possible to take only one harvest during each year. The non-treated sub-plots were mowed early for broad-leaved weed control in 1957. Main plots treated with 2 or 3 lb/A of dalapon yielded significantly more birdsfoot trefoil in 1957 than plots not treated or treated with 8 lb per acre of TCA (Table 15). High yields of birdsfoot trefoil from dalapon-treated plots were due to better control of weed grasses obtained with this herbicide. Yields of birdsfoot trefoil from plots receiving TCA or dalapon in 1958 were significantly better than the check. Broad-leaved weeds were reduced to a negligible amount by 4–(2,4–DB) and mowing in 1957. Significant reductions in broad-leaved weeds by use of 4–(2,4–DB) in 1958, increased yields of birdsfoot trefoil as compared

Table 10. Yields (lb/A oven-dry matter) of a new seeding of alfalfa as affected by post-emergence herbicides in 1957 and 1958 (second cutting).[a]

Main treatment[b]	No treatment			Sub-treatments												Averages of main treatments		
				4–(2,4–DB) alone						4–(2,4–DB) with 2 lb/A of dalapon[c]								
				1 lb/A			2 lb/A			1 lb/A			2 lb/A					
	1957	1958	Av.	1957	1958	Av.	1957	1958	Av.	1957	1958	Av.	1957	1958	Av.	1957	1958	Av.
TCA (8 lb/A)	890	1,940	1,420	1,020	2,090	1,560	860	2,230	1,540	670	1,920	1,300	700	1,870	1,280	830	2,010	1,420
Dalapon (2 lb/A)	820	2,010	1,420	990	2,030	1,510	1,050	2,280	1,670	900	2,000	1,450	960	2,130	1,540	940	2,090	1,520
Dalapon (3 lb/A)	1,000	1,940	1,470	1,090	2,030	1,560	1,120	1,920	1,520	910	1,990	1,450	1,100	1,840	1,470	1,040	1,940	1,490
None	850	1,900	1,380	1,060	2,490	1,780	1,060	2,020	1,540	760	2,210	1,490	800	1,940	1,370	910	2,110	1,510
Average	890	1,950	1,420	1,040	2,160	1,600	1,020	2,110	1,570	810	2,030	1,420	890	1,950	1,420	920	2,040	1,480

LSD 5% Sub-treatments 1957 = 122

[a]Forage was harvested August 23, 1957, and August 22, 1958.

[b]Main treatments with TCA and dalapon were applied either alone or as mixtures with 4–(2,4-DB) of the sub-treatments and June 7, 1957 and on May 26, 1958.

[c]Sub-treatments of dalapon were applied after the first cutting.

Table 11. Yields (lb/A oven-dry matter) of broad-leaved weeds in a new seeding of alfalfa as affected by post-emergence herbicides in 1957 and 1958 (second cutting).[a]

Main treatment[b]	Sub-treatments															Averages of main treatments		
	No treatment			4-(2,4-DB) alone						4-(2,4-DB) with 2 lb/A of dalapon[c]								
				1 lb/A			2 lb/A			1 lb/A			2 lb/A					
	1957	1958	Av.	1957	1958	Av.	1957	1958	Av.	1957	1958	Av.	1957	1958	Av.	1957	1958	Av.
TCA (8 lb/A)	113	46	80	59	7	33	0	12	6	13	5	9	2	0	1	37	14	26
Dalapon (2 lb/A)	264	171	218	13	21	17	0	0	0	41	3	22	16	6	11	67	40	54
Dalapon (3 lb/A)	369	14	192	63	13	38	38	11	25	8	5	7	44	9	27	105	10	58
None	330	165	248	0	17	9	0	0	0	3	0	2	0	0	0	67	36	52
Average[d]	216	99	184	27	14	24	8	6	8	13	3	10	13	4	10	68	25	47

[a]Forage was harvested August 23, 1957, and August 22, 1958.
[b]Main treatments with TCA and dalapon were applied either alone or as mixtures with 4-(2,4-DB) on June 7, 1957, and May 26, 1958.
[c]Supplemental sub-treatments of dalapon were applied after the first cutting. 4-(2,4-DB) was applied June 7, 1957, and May 26, 1958 (about a month before the first cutting).
[d]Analysis of variance was not calculated because of the nature of the data.

Table 12. Yields (lb/A oven-dry matter) of weed grass in a new seeding of alfalfa as affected by post-emergence herbicides in 1957 and 1958 (second cutting.)[a]

Main treatment[b]	Sub-treatments															Averages of main treatments		
	No treatment			4–(2,4–DB) alone						4–(2,4–DB) with 2 lb/A of dalapon[c]								
				1 lb/A			2 lb/A			1 lb/A			2 lb/A					
	1957	1958	Av.	1957	1958	Av.	1957	1958	Av.	1957	1958	Av.	1957	1958	Av.	1957	1958	Av.
TCA (8 lb/A)	740	280	510	700	370	530	920	130	530	2	11	7	10	15	13	470	160	120
Dalapon (2 lb/A)	770	34	400	580	57	320	780	210	500	2	3	3	8	0	4	430	61	240
Dalapon (3 lb/A)	302	75	190	310	250	280	570	510	540	1	26	14	32	11	22	240	170	210
None	1,650	78	860	1,180	190	680	1,180	920	1,050	13	0	7	20	0	10	810	240	520
Average[d]	860	94	490	690	220	450	860	440	650	5	10	7	18	6	12	490	160	320

[a]Forage was harvested August 23, 1957, and August 22, 1958.
[b]Main treatments with TCA and dalapon were applied either alone or as mixtures with 4–(2,4–DB) of the sub-treatments on June 7, 1957 and May 26, 1958.
[c]Sub-treatments of dalapon were applied after the first cutting. 4–(2,4–DB) was applied June 7, 1957, and May 26, 1958.
[d]Analysis of variance was not calculated because of the nature of the data.

Table 13. Yields (lb/A oven-dry matter) of a new seeding of alfalfa as affected by post-emergence herbicides in 1958 (third cutting).[a]

Main treatments[b]	Sub-treatments					Averages of main treatment
	No treatment	4–(2,4–DB) alone		4–(2,4–DB) with 2 lb/A dalapon[c]		
		1 lb/A	2 lb/A	1 lb/A	2 lb/A	
TCA (8 lb/A	1,680	1,610	1,710	1,390	1,610	1,600
Dalapon (2 lb/A)	1,490	1,600	1,800	1,640	1,400	1,590
Dalapon (3 lb/A)	1,580	1,630	1,610	1,410	1,570	1,590
None	1,500	1,660	1,500	1,540	1,170	1,470
Average	1,560	1,630	1,660	1,490	1,430	

LSD 5% Sub-treatments = 137

[a]Forage was harvested September 22, 1958.
[b]Main treatments of TCA and dalapon were applied either alone or as mixtures with 4–(2,4–DB), of the sub-treatments on May 26, 1958.
[c]Sub-treatments of dalapon were applied after the first cutting. 4–(2,4–DB) was applied May 26, 1958.

with the check. Extremely high yields of broad-leaved weeds occurred in 1958 because of heavy rainfall. The use of 4–(2,4–DB) reduced yields of weeds considerably, but even then the yields of weeds remaining ranged from 1,210 to 2,460 lb/A. Dalapon and TCA reduced yields of broad-leaved weeds approximately 50% even though these materials are not regarded as affective on broad-leaved weeds.

In 1957, treatments with 2 or 3 lb/A of dalapon significantly increased the number of birdsfoot trefoil plants as compared with no treatment or treatment with 8 lb/A of TCA (Table 16). Plots treated with TCA had about as many birdsfoot trefoil plants as the check. There were small differences among the birdsfoot trefoil populations on plots receiving TCA and dalapon in 1958, but all treated plots had significantly more plants than the check. Plots treated, in 1957, with 4–(2,4–DB) had significantly fewer plants than plots not treated, indicating that reduction in stand occurred from treatments with 4–(2,4–DB). Plots treated with the 2-lb rate had significantly less plants than the 1-lb rate. In 1958, however, plots treated with 4–(2,4–DB) had significantly more plants than the check plots. Mowing early for broad-leaved weed control in 1957, but not in 1958, may account for some of the differences in response. The more numerous plants in treated plots in 1958 reflect the benefits obtained from the reduction of broad-leaved weed growth with 4–(2,4–DB). Sub-treatments with 2 lb/A of dalapon applied late in addition to 4–(2,4–DB) did not improve the stand of birdsfoot trefoil.

Throughout the experiments, good over-all weed control was generally obtained with combinations of 4–(2,4–DB) and dalapon or TCA. These combinations were especially helpful in establishing stands and increasing yields of seedling birdsfoot trefoil. With alfalfa, however, the control of weed grasses was obtained at the sacrifice of some alfalfa yield with no effect on stand. If the infestation with weed grasses had been more severe, the control with dalapon or TCA might have increased the alfalfa yields. Under conditions of these experiments, the alfalfa was vigorous and the stand thick enough to

Table 14. Number of alfalfa plants in 6 square feet as affected by post-emergence herbicides. Stands determined August 31, 1957 and August 29, 1958.

Main treatment[a]	No treatment			Sub-treatments												Averages of main treatments		
				4-(2,4-DB) alone						4-(2,4-DB) with 2 lb/A of dalapon[b]								
				1 lb/A			2 lb/A			1 lb/A			2 lb/A					
	1957	1958	Av.	1957	1958	Av.	1957	1958	Av.	1957	1958	Av.	1957	1958	Av.	1957	1958	Av.
TCA (8 lb/A)	87	63	75	84	61	73	76	64	70	87	58	73	96	59	78	86	61	74
Dalapon (2 lb/A)	89	65	77	88	63	76	88	59	74	91	56	74	89	62	76	89	61	75
Dalapon (3 lb/A)	89	63	76	95	58	77	87	62	75	100	55	78	98	61	80	94	60	77
None	90	63	77	89	67	78	87	60	74	87	65	76	82	61	72	87	63	75
Average	88	63	76	89	62	76	85	61	73	91	58	75	91	61	76	89	61	75

[a]Main treatments with TCA and dalapon were applied either alone or as mixtures with 4-(2,4-DB) of the sub-treatments on June 7, 1957, and May 26, 1958.
[b]Sub-treatments with dalapon were applied after the first cutting.

Table 15. Yields (lb/A of oven-dry matter) of birdsfoot trefoil and weeds as affected by post-emergence herbicides in 1957 and 1958.[a]

Main treatments[b]	Sub-treatments[c] lb/A 4–(2,4–DB)	Yields of birdsfoot trefoil and weeds								
		Birdsfoot trefoil			Broad-leaved weeds			Weed grass		
		1957	1958	Av.	1957	1958	Av.	1957	1958	Av.
TCA (8 lb/A)	1	230	390	310	120	1,680	900	200	200	200
	2	110	390	250	26	1,410	720	380	460	420
	None[d]	190	10	99	160	5,900	3,030	130	20	74
	Av.	180	260	220	100	3,000	1,550	240	230	230
Dalapon (2 lb/A)	1	340	290	320	120	2,140	1,130	87	21	49
	2	350	490	420	120	2,200	1,160	120	510	320
	None[d]	280	25	150	79	5,050	2,570	43	0	21
	Av.	320	270	290	110	3,130	1,620	83	180	130
Dalapon (3 lb/A)	1	360	260	310	49	2,460	1,250	40	37	38
	2	340	610	480	78	1,210	640	40	160	98
	None[d]	130	39	84	33	5,940	2,990	17	60	38
	Av.	280	300	290	53	3,200	1,630	32	84	58
None	1	150	89	120	280	2,380	1,330	1,340	1,920	1,630
	2	110	99	100	220	2,050	1,140	1,460	2,030	1,740
	None[d]	190	90	140	250	10,330	5,290	480	380	430
	Av.	150	93	120	250	4,920	2,590	1,090	1,440	1,270
Sub-treatment averages	1	270	240	250	140	2,170	1,160	420	540	480
	2	200	400	310	110	1,720	910	500	790	640
	None	200	40	120	130	6,810	3,470	170	120	140
LSD 5% Main treatments		120	ns		90	ns		780	—[e]	
Sub-treatments		62	150		ns	2,360		ns	—	

[a]Forage was cut on July 30, 1957 and July 28, 1958.
[b]Main treatments with TCA and dalapon were applied either alone or as mixtures with 4–(2,4–DB) of the sub-treatments on June 7, 1957 and May 26, 1958.
[c]Because supplemental treatments with dalapon were applied after cutting, only data where 4–(2,4–DB) was used alone are presented.
[d]These sub-treatments were mowed early for broad-leaved weed control in 1957.
[e]Analysis of variance was not calculated because of the nature of the data.

eliminate many of the weed grasses. Birdsfoot trefoil, however, benefited from TCA or dalapon because it was not as vigorous as alfalfa and weed grasses were more of a problem. Dalapon was somewhat superior to TCA for weed control.

Practical control of broad-leaved weeds was obtained with 1 lb/A of 4–(2,4–DB). The 2-lb rate controlled broad-leaved weeds slightly better than the 1-lb rate, but injury to birdsfoot trefoil was generally more severe with the higher rate. Control of broad-leaved weeds in alfalfa was highly successful and yields of nearly 3 tons per acre of alfalfa were obtained during 1958.

Summary

An amine and an ester of 4–(2,4–DB) were compared for effects on legumes and weeds. Rates of ½, 1, and 2 lb/A were used on alfalfa and birdsfoot trefoil. Rates of ½, ¾, 1, 1½ and 2 lb/A were used on red clover. Dalapon at 2 lb/A was also used in combination with 1 lb/A of each formulation. Highest yields of alfalfa were obtained with 1 lb/A of amine. Rates above 1 lb of amine or rates of 1 lb or more of the ester did not increase yields of alfalfa appreciably above the check. Two lb/A of dalapon plus 1 lb/A of 4–(2,4–DB)

Table 16. Number of birdsfoot trefoil plants in 6 square feet as affected by post-emergence herbicides. Stands were determined August 13, 1957, and August 14, 1958.

Main treatment[a]	No treatment			Sub-treatments												Averages of main treatments		
				4-(2,4-DB) alone						4-(2,4-DB) with 2 lb/A of dalapon[b]								
				1 lb/A			2 lb/A			1 lb/A			2 lb/A					
	1957	1958	Av.	1957	1958	Av.	1957	1958	Av.	1957	1958	Av.	1957	1958	Av.	1957	1958	Av.
TCA (8 lb/A)	54	26	40	53	35	44	39	33	36	52	39	46	44	38	41	48	34	41
Dalapon (2 lb/A)	84	19	52	63	36	50	71	34	53	77	35	56	71	36	54	73	32	53
Dalapon (2 lb/A)	80	18	49	87	35	61	82	49	66	87	42	65	85	38	62	84	36	60
None	72	17	45	49	17	33	26	17	22	45	24	35	34	18	26	45	19	32
Average	72	20	46	63	31	47	54	33	44	65	35	50	59	32	46	63	30	46

LSD 5% Main treatments: 1957 = 8; 1958 = 10.
Sub-treatments: 1957 = 4; 1958 = 6.

[a]Main treatments with TCA and dalapon were applied either alone or as mixtures with 4-(2,4-DB) of the sub-treatments June 7, 1957, and May 28, 1958.
[b]Sub-treatments with dalapon were applied after cutting.

reduced yields of alfalfa and red clover as compared with 1 lb/A of 4–(2,4–DB) alone.

Two lb/A of dalapon in combination with 1 lb/A of 4–(2,4–DB) resulted in yields of birdsfoot trefoil about 3 times greater than when 1 lb of 4–(2,4–DB) was used alone.

Red clover was tolerant to all rates of 4–(2,4–DB) and its use resulted in increases in yields ranging from 46 to 96 percent above the check. No reduction in yield occurred from the use of 4–(2,4–DB), but dalapon reduced yields of red clover.

Alfalfa stands were not affected by any of the herbicides. Red clover stands were reduced and trefoil stands increased by the use of dalapon in combination with 4–(2,4–DB).

One and 2 lb/A rates of an ester of 4–(2,4–DB) were compared in combinations with 8 lb/A of TCA, 2 lb/A of dalapon and 3 lb/A of dalapon. Half of the plots received a second application of 2 lb/A of dalapon after the first cutting.

Spraying with 1 or 2 lb/A of 4–(2,4–DB) post-emergence nearly eliminated broad-leaved weeds and increased the yields of the first cutting of seedling alfalfa. Control of broad-leaved weeds and yields of alfalfa were similar with both rates of 4–(2,4–DB). During the dry season of 1957, the removal of broad-leaved weeds from the first cutting increased yields of alfalfa in the second cutting. TCA at 8 lb/A and dalapon at 2 or 3 lb/A reduced yields of weed grasses and also reduced the yields of alfalfa somewhat. Dalapon was less injurious to alfalfa and more effective than TCA for control of weed grasses. Two lb/A of dalapon was less injurious to alfalfa than 3 lb. Supplemental treatments with 2 lb/A of dalapon after the first cutting were effective for controlling weed grasses but reduced the yields of the second cutting of alfalfa. The number of alfalfa plants present was unaffected by treatments.

Yields of birdsfoot trefoil were increased when broad-leaved weeds were reduced with 4–(2,4–DB). Stands of birdsfoot trefoil were slightly improved by treatments with 4–(2,4–DB) where grasses were controlled by early treatment with dalapon at 3 lb/A, but under some conditions treatments with 4–(2,4–DB) resulted in reduced stands.

The 3-lb rate of dalapon was more effective than the 2-lb rate of dalapon or the 8-lb rate of TCA for controlling weed grasses and increasing the yield and stands of birdsfoot trefoil. These herbicides were effective only when broad-leaved weeds were controlled with 4–(2,4–DB).

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Chemical Weed Control in Young Grapes[1]

C. C. Doll[2]

The use of herbicides for controlling weeds in the grape row is becoming resolved in mature vineyards. Oil and DNBP mixtures can be used because of the protective covering of old bark on the trunk (2, 4). Herbicides such as CIPC, monuron and diuron can be used as a band treatment in the row because of the resistance to these chemicals where only a portion of the root zone is treated (1, 3). Most of the above references caution about the use of these materials on young vineyards or transplants.

It is of primary importance that weed control be practiced in young vineyards to reduce competition with the young grape plants. It is as laborious and costly to control weeds in young vineyards as in mature vineyards. If herbicides could be used for weed control during the period while the vines are reaching maturity, it would be of economic importance.

Materials and Methods

Weed control trials on young Concord and Fredonia grapes were conducted at the Bluffs Experimental Fruit Farm near Council Bluffs, Iowa. The soil was an Ida silt loam, a loess soil characterized by its uniformity through great depths and also by the absence or near absence of sand and/or clay particles. Although the soil type runs uniform in the area, the sites selected for each variety varied in previous cropping systems, so that the fertility and weed seed content of the soils differed for each site. All plantings were made on a small terrace with the terrace crown serving as the plant row. The principal weeds were yellow foxtail *(Setaria lutescens)* and kochia *(Kochia scoparia)*. Numerous other annual weeds were present, but only in small numbers. Horsenettle *(Solanum carolinense)* and hedge bindweed *(Convolvulus sepium)* were the only perennials present and these were in small numbers.

Three separate trials were conducted, one with the Concord variety and two with the Fredonia variety. The Concord vineyard was planted in 1956 and treatments were made on April 27, 1957, and May 1, 1958. The experiment was arranged in a randomized block design with four replications. In 1957, plots included three vines eight feet apart. In 1958, two thirds of the 1957 area was given repeat applications with the remaining plot area left to study residual effects. Twenty 4-inch band treatments in the row were made with herbicide rates based on per acre actually treated. Rates per acre of materials applied in 1957 were: 1.6, 3.2, and 4.8 pounds of 3–(3,4–dichlorophenyl)–1,1–dimethylurea (diuron), 8 pounds of isopropyl N–(3–chlorophenyl)carbamate (CIPC), 2 and 4 pounds of 2–chloro–4,6–bis(ethylamino)–*s*–triazine (simazine), and a mixture

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of 3.2 pounds of diuron plus 0.5 pounds of 3–amino–1,2,4–triazole (amitrole). 1958 treatments were the same except that 1.6 pounds of diuron plus 0.5 pound of amitrole were substituted for the 4.8 pounds of diuron and the rates of simazine were reduced to 1 and 2 pounds per acre.

One-year-old wood was pruned and weighed at the end of the growing season in both years. Weed counts were made on July 8, 1958 on both residual and repeat treatment areas, and on October 21, 1958 on the repeat treatment area only. The early season counts were made in a 1- by 2-foot area in the center of each plot. The late season counts were made in a 2- by 3-foot area in the center of the plot.

The two-year test with the Fredonia variety was initiated on April 27, 1957, one day after planting. In this planting, plots included two vines with 10-foot spacing and four replications of each. Repeat applications on the same areas were made in 1958. Materials and rates were the same as for the Concord test except that the mixture of 3.2 pounds of diuron plus 0.5 pound of amitrole was not included. Shoot growth and weed count studies were also the same as made on the Concord planting.

The third trial consisted of four replications of two-vine plots in a Fredonia vineyard planted in 1958. Herbicide applications were made on May 1, 1958, two days after planting. The following materials and combinations were used: 1.6 and 3.2 pounds of diuron; 1.6 pounds of diuron plus 0.5 and 1.0 pound of amitrole; 1 and 2 pounds of simazine; 1 pound of simazine plus 0.5 pound of amitrole; 0.5 and 1 pound of amitrole; 8 and 16 pounds of CIPC. The effectiveness of these herbicides was determined by weed counts on July 8, 1958. Final summation at the end of the year was not possible due to a disturbance of the treatment area by cultural operations shortly after the first count was made. The effect of the herbicides on grape plant growth was determined by linear measurement of the shoot growth at the end of the growing season.

All herbicides were applied with a knapsack sprayer with a three-nozzle boom, using flat spray nozzles and 30 to 40 psi, except the CIPC in 1958, which was applied broadcast as a 5-percent dust. About 100 gallons of water per acre were applied. Sprays were directed in a 24-inch band in the row, with direct contact of the grape vines. Check plot areas received only hand weeding of a 36-inch diameter area around the vine. In all cases except the newly-set Fredonia vines in 1957 and 1958, the vines were completely dormant. The newly-set Fredonia vines had swollen buds, indicating breaking of dormancy. No supplemental water was added either before or after applications. Heavy infestations of foxtail and kochia were present in the seedling stages. The surface soil was dry at the time of application in both years. In 1957, 12 days elapsed between application and rainfall; this was followed by 11.5 inches of rain in May and June and another 11 inches from July through September. In 1958, 7 days elapsed between application and rainfall, and was followed by only 3 inches of rain for May and June and over 20 inches from July through September.

RESULTS AND DISCUSSION

Concords, 1957 and 1958.

Herbicide treatments in 1957 gave much more effective weed control than the same treatments in 1958. At the end of 1957 all rates of diuron and simazine gave satisfactory control. CIPC gave effective weed control for 12 weeks, but was ineffective thereafter. A mottled chlorosis of the leaves appeared on all vines treated with diuron or simazine. This was first visible on July 1. It persisted until the end of the season on vines treated with the higher rates of diuron and both rates of simazine. Vines in the 1.6 pound diuron treatments regained normal appearance by the end of the growing season. Growth of vines as determined by weight of wood showed no significant differences between treatments and the check (Table 1). CIPC-treated vines made the least shoot growth in 1957 and again in 1958.

Table 1. Weight of shoot growth of young Concord and Fredonia grapes in 1957 and 1958 weed control studies.

Herbicide	Rate lb/A	Av. weight, oz., shoots per vine			
		Concord[a]		Fredonia[b]	
		1957	1958	1957	1958
Diuron	1.6	6.2	15.6	1.6	4.5
Diuron	3.2	4.8	13.0	1.0	4.5
Diuron	4.8	9.5	—	—	—
Diuron+amitrole	1.6+0.5	—	27.8	1.4	7.5
Diuron+amitrole	3.2+0.5	7.9	15.2	—	—
CIPC	8.0	4.2	7.9	1.0	2.8
Simazine	1.0	—	16.0	—	3.5
Simazine	2.0	4.8	12.0	1.2	5.5
Simazine	4.0	5.4	—	1.3	—
Check	—	6.2	16.4	1.2	2.5
LSD at 5% level		4.6	15.4	0.4	3.8
LSD at 1% level		6.2	20.9	0.6	5.2

[a]Planted in 1956.
[b]Planted in 1957.

Weight of the 1958 shoot growth also showed no significant differences between herbicide treatments and the check. In most cases, the shoot growth was about equal to that of the check.

1958 weed control results showed acceptable control only with the use of diuron, alone or in combination with amitrole (Table 2). The reduction of simazine to 1 and 2 pounds in 1958 was successful in reducing grape leaf chlorosis, but did not succeed in controlling weeds. Since the 2-pound rate was used in both years, this indicates the effect that weather may have on herbicides. The 2-pound rate was more effective than the 1-pound rate. Weed counts on July 8 and October 21 both showed significant reductions of weed numbers for all treatments. However, the weed populations in the CIPC and simazine plots were too great to be considered practical control at the rates used. They also allowed an increase of weed numbers late in the season.

Table 2. Weeds per square foot in herbicide plots in young Concord grapes, 1958.

Herbicide	Rate lb/A	Weeds per sq. ft.			Per cent control Oct. 21
		1957 residual area	1958 treatment area		
		July 8, 1958	July 8	Oct. 21	
Diuron	1.6	12	2	2	96
Diuron	3.2	13	1	2	97
Diuron	4.8	8	—	—	—
Diuron +amitrole	1.6+0.5	—	1	3	94
Diuron +amitrole	3.2+0.5	3	1	1	99
CIPC	8.0	24	17	33	34
Simazine	1.0	—	37	41	17
Simazine	2.0	44	9	34	33
Simazine	4.0	27	—	—	—
Check	—	47	74	50	—
LSD at 5% level		14.0	19.2	6.8	—
LSD at 1% level		20.4	26.2	9.3	—

There were residual effects from all the 1957 treatments through July 8, 1958, as determined by weed counts. However, only the 4.8 pound rate of diuron and the 3.2 pounds of diuron plus 0.5 pound of amitrole resulted in a sufficient reduction of weed numbers to be considered good control on this date. Weed counts were not made in the residual area at the end of the 1958 season because visual observations indicated no control at that time.

Fredonia, 1957 and 1958.

As with the Concords, the treatments in 1957 gave satisfactory control throughout the season except for CIPC, which was effective only through July. Chlorosis and mottling of the leaves of Fredonia was similar to that of Concord. Simazine at 2 and 4 pounds per acre appeared to cause a greater degree of chlorosis than did diuron. However, little difference occurred between these treatments when growth measurements were taken at the end of the season (Table 1). Vines in the 1.6-pound rate of diuron plots were free of injury symptoms at the end of the growing season, and made the greatest increase in growth as compared to the check vines. Some stunting of the plants in the CIPC plots was visible in June. This might have resulted from the contact of the CIPC solution with the swollen vegetative buds at the time of application. Growth of the vines was near normal at the end of the season and no significant differences in the amount of growth were determined.

In 1958, the amount of shoot growth made by the 2-year-old grape vines in the check areas and the CIPC-treated areas was low and indicated that competition from weeds was more detrimental to growth than the herbicides used. In this year, only the combination of 1.6 pounds of diuron and 0.5 pound of amitrole resulted in significant increase in shoot growth over the check treatment.

The weed population in this vineyard in 1958 was considerably less than in the Concord area and the herbicides, other than diuron, were somewhat more effective on a percentage basis (Table 3). How-

Table 3. Weeds per square foot in herbicide plots in 2-year old Fredonia grapes, 1958.

Herbicide	Rate lb/A	Weeds per sq ft		Per cent control Oct. 21
		July 8	Oct. 21	
Diuron	1.6	3	5	82
Diuron	3.2	2	1	96
Diuron +amitrole	1.6+0.5	1	2	92
CIPC	8.0	9	16	44
Simazine	1.0	15	16	41
Simazine	2.0	8	11	59
Check	—	29	27	—
LSD at 5%		7.4	5.4	
LSD at 1%		10.1	7.4	—

ever, diuron at 1.6 pounds, alone or with 0.5 pound amitrole, or at 3.2 pounds, is the only herbicide that can be considered to have given good control. The addition of amitrole did not materially improve the control obtained. CIPC and simazine reduced the number of weeds significantly on both dates that counts were made. However, the percent control was poor. There were few new weeds after July in this vineyard.

Fredonia, 1958.

Reductions in weed numbers were significant for early season weed control in newly planted Fredonia grapes in 1958 (Table 4).

Table 4. Weed control and shoot growth studies on Fredonia grapes with post-planting application of herbicides, 1958.

Herbicide	Rate lb/A	Weeds/sq ft July 8	Per cent control July 8	Inches of grape shoot growth, Oct. 21
Diuron	1.6	4	81	90
Diuron	3.2	1	97	79
Diuron +amitrole	1.6+0.5	5	76	60
Diuron +amitrole	1.6+1.0	4	80	63
CIPC	8.0	9	55	38
CIPC	16.0	4	78	39
Simazine	1.0	15	21	60
Simazine	2.0	6	83	60
Simazine +amitrole	1.0+0.5	8	60	45
Amitrole	0.5	13	30	40
Amitrole	1.0	19	0	35
Check	—	19	0	27
LSD at 5% level		6.0	—	42.1
LSD at 1% level		7.7	—	54.3

Weed counts on July 8 showed good control for all plots receiving diuron at 1.6 and 3.2 pounds, CIPC at 16 pounds or simazine at 2 pounds. The use of amitrole alone or in combination with diuron or simazine did not reduce the number of weeds.

There was a great variation in the amount of shoot growth made in 1958 by these young Fredonia vines. Short growth on the vines in the check plots can be partially attributed to competition with weeds. All treatments resulted in greater vine growth than the check, although only the 16 and 3.2 pounds of diuron were significantly

greater. There was a trend in shoot growth reduction where amitrole was applied, although not significant in amount. A stunting of the vines during the early part of the season was noted in the CIPC plots. This was similar to the injury noted on post-planting applications to Fredonia in 1957. As in that year, vine growth was normal by the end of the season.

SUMMARY

1. Weed control studies were conducted on young Concord and Fredonia grapes over a two-year period.
2. Diuron at 1.6, 3.2 and 4.8 lb./A was an effective herbicide in both years. Increased effectiveness and longer residual action resulted from the higher rates. Chlorosis of grape leaves was observed from all rates in 1957 but not in 1958.
3. Simazine at 2 and 4 lb./A was an effective herbicide in 1957, but caused considerable chlorosis of the grape leaves. Reduction of the application rates to 1 and 2 pounds in 1958 reduced the amount of plant injury and also the degree of weed control. Seasonal variation was more noticeable with simazine than with diuron.
4. CIPC at 8 and 16 pounds per acre gave a significant reduction of weed numbers when compared to the untreated check plots, but the degree of control for the full season was not acceptable. It lost its effectiveness about 12 weeks after treatment.
5. Amitrole at 0.5 and 1.0 pounds per acre, applied on May 1 before weeds germinated, failed to reduce the weed population significantly.
6. Residual weed control was effected with 3.2 and 4.8 pounds of diuron through mid-season of the second year. This period was characterized by below normal rainfall.
7. No differences in variety reaction to the herbicides was noted.
8. Shoot growth was not significantly reduced by any herbicide treatment.

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Herbicidal Value of Arsenic Trioxide in Eastern United States[1]

ALBERT L. LEAF and ROBERT E. SMITH, JR.[2]

THE use of various arsenic compounds as herbicides to control undesirable vegetation, reducing fire hazards and allowing convenient access, has long been recommended.

Arsenic trioxide, due to its relatively low solubility, has a longer toxic effect to vegetation than sodium arsenite (7). Soil properties affect the rate of arsenic fixation or tie-up in soil in unavailable form to plants, the depth of penetration of arsenic in soil (8) and the period of time the arsenic remains toxic to plants. Sandy soils fix the least arsenic and allow deepest penetration into the soil, while clayey soils fix the greatest proportion of arsenic and restrict depth of penetration into the soil (1, 3). Thus higher rates of application of arsenic are necessary in fine textured soils to obtain the same toxic effect as in coarse textured soils (4). Since arsenic trioxide tends to remain near the surface, Ikenberry et al. (5), noted that this herbicide was effective on shallow-rooted annual plants. Moisture is an important environmental factor affecting the solubility and penetrability of arsenic into the soil (6).

The purpose of this paper is to report on the herbicidal value of three forms of arsenic trioxide (As_2O_3) on experimental sites in eastern United States.

FORMS OF As_2O_3 AND RATES OF APPLICATION

This project, initiated in 1955, involved a minimum of three rates of application of various forms of As_2O_3 at each experimental site, applied broadcast. The forms of As_2O_3 included crystalline 99.4% As_3O_2, crude powder 92.0% As_2O_3, and crude powder 92.0% As_2O_3 plus 2% $CaCl_2$. The rates of application ranged from 450 to 1650 lb/A elemental As as crystalline form, 475 to 1025 lb/A elemental As as crude powder form, and 725 to 2250 lb/A elemental As as crude powder plus $CaCl_2$ form. These rates of application were determined from the report by Crafts and Buck (2) and were higher than the minimum rates recommended for California conditions.

The crystalline material was easy to apply broadcast by hand or lawn seeder. However, due to the granule-size particles, distribution was not entirely uniform and solubility was less than the powder forms. The minimum rate of application of the crystalline As_2O_3

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for uniform distribution is 1200 lb/A since lower rates tend to leave considerable distance between crystals on the ground. This form was applied to plots at three sites with soils of loamy sand, loam, and silt loam textures in Pennsylvania, North Carolina and South Carolina. Only on loamy sand soil was there evidence of herbicidal effect with As_2O_3 treatments up to 2000 lb/A. This effect on grass vegetation decreased with time so that three years after treatment the effect was very slight. On two of the sites traces of crystalline As_2O_3 were still visible three years after treatment.

The crude powder material lends itself to uniform distribution by hand and lawn seeder. The minimum rate of application of the crude powder As_2O_3 for uniform distribution is 700 lb/A by hand or 1000 lb/A by seeder. The handling of the crude powder is quite hazardous since the dry powder tends to blow in the slightest wind. Contact of the powder with the skin results in irritation and eventually blistering which generally requires about a week to clear. Because of handling difficulties, this powder was applied on only one site, located in New York. On a loam soil treated with up to 1500 lb/A, no herbicidal effect was noted on grass vegetation at three months, one and three years after treatment.

The crude powder plus 2% $CaCl_2$, called the non-dusting form because it does not blow unless application is made during a strong wind, lends itself to distribution similar to the powder form. There is a tendency for this material to lump in the seeder hopper causing skips. There was no burning or irritation to the skin after handling the non-dusting powder. However, if excessively handled, the $CaCl_2$ causes a drying of the skin.

FIELD EXPERIMENTATION

Description of areas.

On each of three sites, two or more series of 1/20 to 1/30 acre plots were treated with three or more rates of non-dusting powder form of As_2O_3. The arsenic was applied in ten pound lots on predetermined sub-units of the plots to aid in uniform distribution. Buffer strips of at least 15 feet separated the plots. Plots were established in New Hampshire, West Virginia and Georgia.

The New Hampshire plots were located on coarse to medium soils of glacial till origin in rolling topography with spruce-fir boreal forest type vegetation and cool, humid, temperate climate. Series I and II plots were established on shallow loam or sandy loam soil over a compacted, clayey layer at approximately one foot, and the soil was wet and mottled almost to the surface. Series III plots were on a deep sandy loam, stone-free and well-drained. The soils ranged in clay content from about 3 to 8.5% and in reaction from pH 4.0 to 4.8. Series I vegetation consisted largely of sedge *(Carex* sp.), horsetail *(Equisetum* sp.), raspberry *(Rubus* sp.) and other herbaceous species, and paper birch *(Betula papyrifera)* and sugar maple *(Acer saccharum)* seedlings. Series II included raspberry, aspen *(Populus tremuloides)*, cherry *(Prunus serotina)*, and willow *(Salix nigra)*, and

some balsam fir *(Abies balsamea)* reproduction; small openings consisted of bracken fern *(Pteridium* sp.), sedges and mosses. Series III was cleared of vegetative cover by a bulldozer immediately prior to establishment of plots.

The West Virginia plots were located in the Appalachian Mountains on soils of the Ashby and Buchanan series developed from shales and sandstones supporting mixed hardwood forest vegetation. The Ashby soil was shaley, shallow, residual, Grey-Brown Podzolic with slow internal drainage. In Series I and II, it had a loam to sandy loam texture with a clay content of 6.8 to 12.4%, a hardpan at one foot depth, and a pH of 4.5 to 5.7. The Buchanan soil was gravelly, shallow, colluvial, Grey-Brown Podzolic with poor internal drainage. In Series I and II, it had a clay loam to sandy loam texture with a clay content of 12.0 to 38.4%, large shale fragments close to the surface, and a pH of 4.5 to 5.7. Series I vegetation on both soils consisted of sprout and seedling growth of basswood *(Tilia americana)*, sycamore *(Platanus occidentalis)*, red maple *(Acer rubrum)*, witchhazel *(Hamamelis virginiana)*, as well as grasses and some herbaceous plants. All Series II plots had been mowed, but sprouts of the woody species were present with herbaceous plants and grasses.

The Georgia plots were located on the Atlantic Lower Coastal Plain, characterized by soils derived from water-laid deposits of sand, relatively level topography, and longleaf-slash-loblolly pine *(Pinus palustris, P. elliottii,* and *P. taeda* respectively) forest vegetation. The soils of the three series of plots were sands with clay contents of 2.6 to 3.0% and reaction of pH 3.6 to 4.5, but variable in depth to gley horizon. Series I soil was a deep sand with no gley horizon within five feet of the surface while Series II and III sands overlaid an impervious layer and gley horizon at about two feet. Series I and II plots consisted of various oak species including blackjack oak *(Quercus marilandica)*, black gum *(Nyssa sylvatica)*, palmetto *(Sabal palmetto)*, gallberry (*Ilex* sp.), blueberry (*Vaccinium* sp.), slash pine seedlings and saplings, longleaf pine in 'grass' stage, and some grasses. Series III consisted largely of a mixture of grasses, with some oaks, palmetto, and few slash and longleaf pine seedlings.

Herbicidal effectiveness of non-dusting As_2O_3.

The three series of New Hampshire plots were treated with 800 to 2200 lb/A As_2O_3. At two months the kill of raspberry was striking on Series I. At ten months after treatment, the Series I plots treated with 1000, 1600 and 2200 lb/A As_2O_3 had vegetation densities of 50, 40 and 10%, respectively, compared to the adjacent non-treated areas. This reduction in cover was in herbaceous plants; the woody vegetation was not injured. In Series II, 1000 lb/A As_2O_3 resulted in kill of mosses, 1600 lb/A greatly reduced raspberry and balsam fir seedlings, and 2200 lb/A killed all moss, fern, sedge and balsam fir seedlings. The hardwoods showed no signs of injury. In Series III, even at 800 lb/A As_2O_3, the establishment of vegetation was negligible, while the non-treated area had a cover of grasses and herbs.

Three years after treatment no herbicidal effect was evident on Series I and II, except for a slight reduction of moss, sedge, fern and herbaceous plants at 2200 lb/A. On Series III, initially free of vegetation, 800 lb/A showed definite retardation in establishment of plants and the 1200 and 1600 lb/A treatments still had 70–80% and 95–99% of the plot surface bare.

The two series of West Virginia plots were treated with 1000 to 2500 lb/A As_2O_3. At three months the series on Ashby soil showed all grasses and herbaceous plants killed but woody plants uninjured. On Buchanan soil some kill of grass and herbaceous plants, and injury to maple and sycamore sprouts was evident. One year after treatment on Ashby soil, grasses and herbaceous plants plus some witchhazel were reduced with no effect evident on the tree species. On the Buchanan soil of Series II (mowed) there was reduced height and density of surviving plants on all treatments, while on Series I no herbicidal effect was noted at 1000 lb/A and only slight kill of grasses and herbs at higher rates. The more moist, poorly drained parts of the plots showed a greater effect than the remainder. Three years after treatment no herbicidal effect on Buchanan soil was evident, except for the wetter portions of Series II. On the Ashby soils at 2000 and 2500 lb/A, irrespective of mowing treatment, 60 to 95% of the surfaces of the plots were bare, but the woody species remained.

The three series of Georgia plots were treated with 1000 to 2200 lb/A As_2O_3. At three months there was complete kill of grass and pine seedlings on all Series I and II plots. At 1000 lb/A, 50% of the woody vegetation was killed; at 2000 lb/A, almost 100% was killed. On Series III, mainly grass vegetation at 800 lb/A many of the grasses were resprouting; at 1200 lb/A 75% of the grasses and all pine seedlings were killed with no effect on the oaks and palmetto; at 1600 lb/A all grasses and pine seedlings plus 20% of the oaks were killed, no effect on palmetto. After three years, on Series I, at 1000 lb/A, the grasses were reduced 80–90%, the black gum and palmetto were weakened, but the longleaf pine were unaffected. At 1600 and 2000 lb/A, all grasses and 50–60% of the oaks were killed, palmetto weakened, long-leaf pine unaffected, and about 50–60% of the plot areas were bare. On Series II, a moist site, at 1200 lb/A, all grasses, most of the woody plants and 90–95% of the palmetto were killed, about 90% of the plot area being bare. At 1800 lb/A and 2200 lb/A, almost 100% of plot areas were bare. On Series III at 800 lb/A, the mixed grasses were reduced 20–30%; at 1200 lb/A, 70–80%; at 1600 lb/A, the highest rate of As_2O_3 in the series, 80–90% of the grasses were killed, while palmetto and some oaks were unaffected, resulting in 80–90% bare surface on the plot.

The results of field trials were compared with greenhouse pot culture trials using surface soil samples from the field plots. Tomato (Prichard or Scarlet Topper variety) and Monterey pine *(Pinus radiata)* were used as indicator species. The results of the pot culture tests paralleled field observations.

SUMMARY

The herbicidal effectiveness of As_2O_3 in Eastern United States varied with type and rate of arsenic application, type of vegetation, and soil moisture, drainage and texture. The toxic effect of non-dusting powder As_2O_3 was greater for grasses and herbaceous plants than woody vegetation three years after treatment. The toxicity of As_2O_3 was greater on coarse than fine textured soils and on wet compared to well-drained soils (Table 1).

Table 1. Degree of herbicidal effect three years following treatment, and minimum effective rate of non-dusting powder As_2O_3, on New Hampshire, West Virginia and Georgia plots.

Area	Vegetation	Soil	Herbicidal effect[a]	Minimum effective rate of As_2O_3+2% $CaCl_2$ lb/A	Remarks
New Hampshire	Herbaceous-woody	Acid, sandy loam	Slight	2200	Slight kill of grass and herbaceous vegetation
	None, (bare soil)	Acid, sandy loam	High	1200	70–80% plot area bare of vegetation.
West Virginia	Woody-herbaceous	Acid, loam to clay loam	Slight	2000	Slight kill of herbaceous vegetation, patchy effectiveness.
		Acid, loam to sandy loam	High	2000	60–95% plot area bare of vegetation, woody vegetation least affected.
	Woody-herbaceous (mowed)	Acid, loam to clay loam	Moderate	2000	Patchy effectiveness, greater toxicity on wetter or poorly drained portions of plots.
		Acid, loam to sandy loam	High	2000	60–95% plot area bare of vegetation, woody vegetation least affected.
Georgia	Dense woody and grass	Acid, sand	High	1000	Most positive toxicity of this study, 95–100% plot area bare of vegetation.

[a]Herbicidal effect more pronounced on wet or poorly drained sites.

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Comments on Tests of Herbicide Mixtures[1]

DONALD P. GOWING[2]

ONE of the important areas of current herbicide research is the investigation of herbicide mixtures. It is a common field experience that use of a single material—even of a "broad-spectrum" herbicide—exerts a selective pressure on the field population because of differential effectiveness on the various weed species. There is some evidence that a part of this derives from purely mechanical considerations (e.g., the spraying system in use may not reach plants of different growth habit equally well), but in any case the weed control program often appears to decrease in effectiveness after several seasons. In addition to favoring the survival of particular species, the non-eradicatory application of even a highly efficient chemical apparently aids in the establishment of populations of individuals which are physiologically most tolerant of the herbicide used. It will probably be granted, then, that the use of mixtures of toxicants will provide more effective control of populations of mixed weed species, and may also reduce the numbers of individuals and species exceptionally tolerant of the herbicide program, especially when the toxicants kill by affecting different physiological processes.

Two points of particular interest in tests of herbicide mixtures are (1) whether or not there is any interaction in the responses to the herbicides which would make their combination unexpectedly effective, i.e., synergistic, and (2) how to determine the "expected" effects. In respect to the first point, and to take the simplest case, the relative effectiveness of two chemically similar toxicants may readily depend on the relative affinities for both the site of toxicity and the site of some mechanism of detoxification. These affinities and the ratios of the toxicants used in the mixture will condition the competition at each site, and if the less toxic material "spares" the destruction of the more toxic material, synergism will be obtained. A complication arises in respect to the second point, the determination of the "expected" effect. This can only occasionally be established by simple summation of the responses to the chemical used separately. When one chemical has already accounted for certain individuals, the other may appear either more or less effective than when used alone, depending, among other things, on the level of kill being tested and on the degree to which susceptibility to the two toxicants is related. (Susceptibility to two quite dissimilar toxicants may be related. If, for instance, one affects permeability and the other respiration, they both might be most effective against weeds in full sunlight, the one because of a connection with transpiration, and the other because of a connection with temperature.) Consequently, demonstration of a real interaction—of synergism or antagonism—between toxicants may not be a simple matter.

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DIRECT PLOT OF PERCENT RESPONSE VS. CONCENTRATION

An early approach to the investigation of interaction in herbicide mixtures was that of A. S. Crafts of the University of California at Davis. He and his co-workers looked for interactive responses to arsenic, boron, and chlorate. They were particularly interested in soil sterilization effects of these chemicals in California soils, and typical results of one of these experiments are shown in Figure 1 (data of Crafts and Cleary, 6).

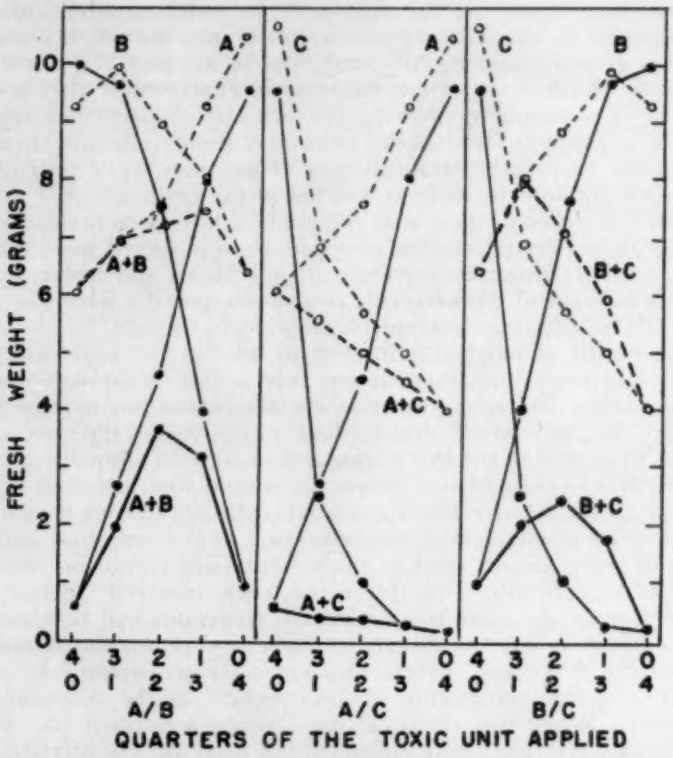

Figure 1. Toxicity of arsenic (A), borax (B), and chlorate (C) to Kanota oats in Yolo clay loam. Rates in quarters of the toxic unit required to inhibit growth to the extent of 90% (solid lines) or 50% (dash lines). Data of Crafts and Cleary, redrawn from Hilgardia 10:401–413.

	Toxic units, ppm dry soil		
	Arsenic	Borax	Chlorate
90% inhibition	540	540	540
50% inhibition	300	300	100

In this test, arsenic (As_2O_3 as sodium acid arsenite), borax ($Na_2B_4O_7 \cdot 10\ H_2O$), or sodium chlorate ($NaClO_3$) were mixed with Yolo clay loam in amounts which would bring about a 50% or 90% suppression of plant growth when used separately. Quarters of these

amounts of the chemicals, two at a time, were then applied to soil in the ratios 4:0, 3:1, 2:2, 1:3, or 0:4, the pots seeded uniformly to Kanota oats, and growth of plants determined after an appropriate period. The hypothesis was that since a total dosage of four quarters of mixed toxicants was used in each combination, points representing the growth of plants exposed to the various combinations should plot as a straight line when the effects were strictly additive. If the lines were concave downward, antagonism between chemicals would be demonstrated. If the lines were concave upward, synergism between chemicals would be demonstrated. They stated that they could demonstrate no synergism between the three chemicals used, but that there was an obvious antagonism of borax for either arsenite or chlorate.

Calculation will reveal that the results from the borax and arsenic combination showed *no* antagonism, if one adds the expected toxicities instead of the dosages. For example, if X is the percent growth suppression by the more toxic agent, and Y is the percent growth suppression by the less toxic agent, then $X + (100 - X)Y$ is the expected combined toxicity.

One applies this to the arsenic: borax combinations by substituting the toxicities actually obtained at the dosages of A and B when used separately. At the 50% growth level, the expected toxicities are 40, 30, 33, 26, and 37% (growth of about 6, 7, 6.7, 7.4, and 6.3 grams). At the 90% level of growth suppression, they are 93, 80, 67, 68, and 91% (growth of about 0.7, 2, 3.3, 3.2, and 0.9 grams). These agree very well with the values obtained, and in fact the mixtures did plot more or less as straight lines in tests with two other soils, there being no evidence of antagonism for borax and arsenic in Fresno sandy loam or Stockton adobe clay.

The results with boron and chlorate, showed, however, clear evidence of antagonism in all three soils.

Crafts and Cleary also pointed out that, particularly at the 90% suppression level, the curves of the chemicals used alone were different—concave downward for borax, almost linear for arsenic, and concave upward for chlorate. Some information on these chemicals can be found from examination of Figure 2, the log-probability plots (data from Crafts, 5, and Crafts and Raynor, 7). The utility of such plots has been pointed out earlier (Gowing, 13). The dosage-response lines thus obtained furnish permissive, although not definitive, information on modes of action of the toxicants, and non-linear ("broken-line") data are interpreted as evidence of more than one mechanism of toxicity.

All three chemicals showed a "break" in the dosage-response line, although the data for arsenic were less definitive than the data for the others. Chlorate was considerably more potent below its "break" point than borax or arsenic for equal dosages. Referring again to the experiment of Figure 1, account was taken of this at the 50% suppression level (the investigators used 100 ppm chlorate and 300 ppm borax or arsenic), but not at the 90% suppression level (they used 540 ppm of all three). When 540 ppm of all three chemicals was used,

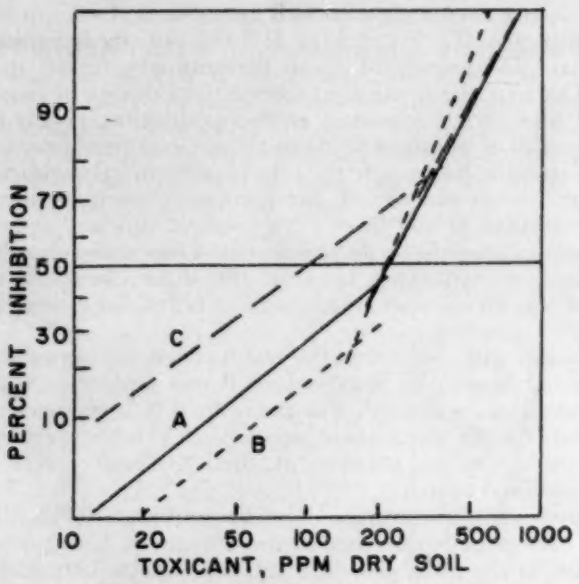

Figure 2. Inhibition of growth of Kanota oats growing on Yolo clay loam mixed with arsenic (A), borax (B), or sodium chlorate (C). Log-probability (probit) plot. Data of Crafts (5), and Crafts and Raynor (7).

the first quarter (135 ppm) of borax and arsenic was much less effective than the first quarter of chlorate. And the more toxic mechanism of borax and arsenic had come into play for the second quarter (270 ppm) of these chemicals, but became operative only for the third quarter of chlorate, by which time the toxicity of this chemical was already at the 85% level. As Crafts and Cleary found, a more nearly uniform relationship of toxicity to dose for the three chemicals was shown at the 50% suppression level, since 25 ppm of chlorate (the first increment) was more nearly equal in toxicity to 75 ppm (the first increment) of the other two materials, and so on.

Let it be clearly stated that these investigators performed very useful work in these studies nevertheless. For, since the borate ore colemanite was very cheap in comparison to chlorate, their work demonstrated that an effective but inexpensive combination was that of a little chlorate and a lot of borax. The point to be made here, then, is not that the direct approach is useless, but that it may sometimes be misleading. Unless one is certain that the toxicants are acting similarly at the levels tested, and that the one may be substituted for the other in a constant fixed ratio, it appears safer to add probable toxicities rather than dosages.

Sheets and Leonard (19) recently reported in WEEDS on mixtures of amitrol with several chemicals. Their design was similar to that of Crafts and Reiber (8). They stated that the responses of barley and kidney beans to mixtures of amitrol and dalapon were about

additive, and data from their Figure 1 substantiate this. However, in another experiment with the commercial formulations of amitrol and dalapon, the data of their Table 1, shown here as Figure 3, indi-

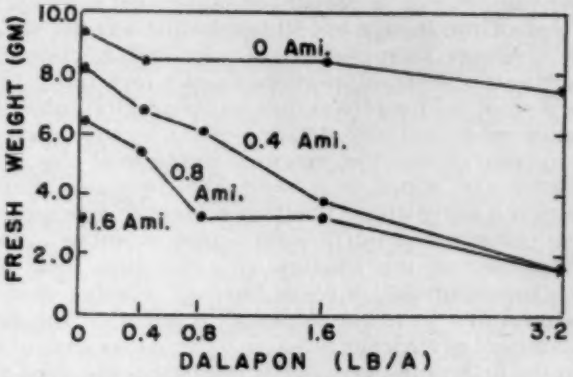

Figure 3. Toxicity of foliar applications of dalapon and amitrol and combinations of the two to barley. Data of Sheets and Leonard 19).

cate that on barley at least the response to the mixture was more than additive. Note the difference in slope of lines with and without amitrol. Two points may be made. First, since the two chemicals show different regressions of response to dose, the least significant difference (LSD) between responses to mixtures is best computed between rates of dalapon, say, at *one* rate of amitrol, or vice versa. And second, it is not necessarily to be expected that the slope for 0.8 lb amitrol per acre will be different from that for 0.4 lb/A; it has been shown (Gowing, 13) that a little amitrol may be very nearly as effective as a great deal more.

In the Table 3 of Sheets and Leonard, the effect of the mixture of amitrol and dalapon appeared more than additive on both barley and kidney beans. Differences in formulations may then be added to their list of reasons why other workers obtained different results. Moreover, although their statement in connection with their data on mixtures of amitrol and monuron was that the effect of the mixture on barnyard grass was approximately additive, calculation of the "expected" response levels will show that the effect appeared in fact more than additive, as was the case with barley and kidney beans. A further part of the reasons for failure of investigators to agree on interpretations may then be laid to some disagreement on what results were obtained.

It is difficult to derive "expected" responses from arithmetic plots of herbicide mixtures when the regression of response on dose is not linear. An exception to this would be found where one element of the mixture has no toxicity at the rate used. Thus there will probably be little question that their most interesting results with mixtures of carboxylic acids (non-toxic at the rates used) with amitrol indeed represented responses greater than merely additive.

Burchfield and Wilcoxon (4) used a fruitful extension of the method of Crafts and co-workers in tests of insecticides. The insecticide work was with the quantal (kill-or-no-kill) response, rather than quantitative (suppression of growth) response, and involved plotting the reciprocal of the dosage for 50% response against the fraction of toxicant. However, their plotted point for each fraction (i.e. ratio) derived from tests of several concentrations for each ratio. These data were then plotted on log-probability paper to establish the LD 50 (amount required to kill 50% of the population) for each fraction, and the reciprocal of this dose was used in the final plot. If the line drawn through the points is straight, this indicates similar joint action, whereas a curve above this line represents synergistic action, and a curve below independent joint action or antagonism.

This adaptation of the response–concentration plot takes into account two important principles of bioassay, whether of single toxicants or of mixtures. First, the approach is based on the determination of the amount of toxicant to attain a particular level of response, rather than the determination of what response is obtained at a given level of toxicant. And second, the response level selected for critical examination was 50%, rather than some arbitrary "maximum kill". Application of both these principles greatly increases the accuracy of a given assay (see Horsfall, 15). However, Bliss (3) has recently noted that the researcher may be content with assays at, say, the 95% or 99% level if the conditions of test exactly parallel those of the intended use. Moreover, those of us in herbicide research have long recognized that principles derived on quantal considerations (all-or-none) are not always strictly applicable to our work. We are often content with a suppression of weed growth short of death, and it has often seemed that herbicide-injured foliage is more subject to destruction by wind, weather, and other accidental agencies than healthy foliage. These accidents even seem to occur often enough to be of practical importance in the assay of the herbicides! Thus, the field plot test will probably always have its place, although the data obtained from it may be rather less precise than required for the elucidation of the principles relating to herbicide interaction.

Storrs and Burchfield (22) also used a preliminary plot on log-probability paper in overcoming another difficulty in toxicological work. The primary reaction of a toxicant may be reversible—reversible inhibition of a vital enzyme system, say—and only if the effect is continued sufficiently long does death result. That is, the time factor is important. Death is then a secondary consequence, but is taken as a measure of the primary reaction. Although "for practical purposes" this is a satisfactory index of the action of the toxicant, the confounding of the secondary with the primary effect has also contributed its share to misunderstanding. Storrs and Burchfield's technique involved plotting time for 50% response to an insecticide mixture vs. fraction of a toxicant, and referred to quantal data.

There are statistical reasons why these techniques cannot be used directly on quantitative data on reduction of stand of weeds, etc., but the principles are worth considering in those tests of herbicide

mixtures in which any conclusions are drawn as to mode of action and interaction of the toxicants. Moreover, the utility of the log-probability plot in herbicidal work has been but very little exploited.

PLOTS OF PERCENT RESPONSE VS. LOG DOSE

One of the rare uses of log plots in herbicidal literature is that of Stone and Stahler (21). They derived linear regression equations for toxicity of solutions of boron and chlorate herbicides to several plants growing in solution culture on vermiculite. They used three concentrations, 10, 100, and 1000 ppm—a range over which Crafts and co-workers (and the author in unpublished work) obtained non-linear plots on either an arithmetic or log-concentration basis. One wonders whether or not the use of only three concentrations may have masked a non-linearity in their data.

Stone and Stahler concluded, as a result of their studies, that Crafts' field recommendations, implying that a unit of boron trioxide was equivalent to a like unit of sodium chlorate, were justified. However, their experimental evidence does not appear to support this conclusion, except possibly at 100 ppm. At 10 ppm, sodium chlorate appeared to be more efficient than boron trioxide, and at 1000 ppm, the reverse was true. This situation obtained with regard to beans, zinnias, alfalfa, and sweetclover, and it was actually only in work with oats that chlorate and boron trioxide were equivalent over the range of concentrations tested.

Their data are reproduced in Figure 4. Compounds B and C, which were mixtures of sodium chlorate and sodium polyborates,

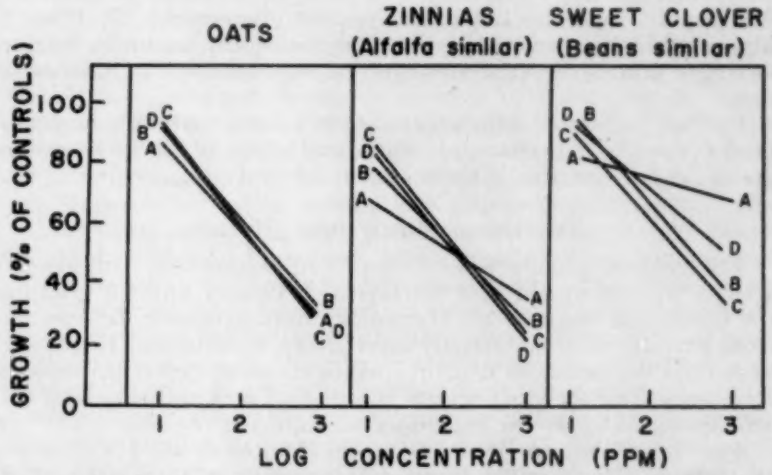

Figure 4. Toxicity of sodium chlorate (A), boron trioxide (D), and mixtures of the two (B and C) with 75 or 60% sodium polyborate contents respectively. Seedlings grown on vermiculite watered with solutions of herbicides at concentrations shown. Data of Stone and Stahler (21—reproduced by permission of Agr. Chem.)

clearly behaved more nearly like boron trioxide—the more toxic constituent—than like chlorate. Only if the investigators averaged the results for all concentrations could the toxicants possibly be considered equivalent in potency. But toxicity of chlorate appears to have a substantially different regression from that of the boron herbicides, such that averaging the results for all rates to obtain an overall comparison between compounds scarcely seems justified. Such a procedure ignores the interaction of "chemical X rate" which their analysis of variance must have shown them (the results were subjected to this statistical procedure). The chemicals are properly compared only with reference to particular rates.

In the opinion of the author, Crafts' fully valid field recommendations for application of chlorate or boron trioxide (as borax) at 250–650 ppm dry soil were based more on the capacity of California soils to fix or inactivate boron, so even if it is of the higher inherent toxicity shown by the data, at least as much boron trioxide as chlorate was required for field use.

However, plotting log dose vs. percent response often provides a very satisfactory transformation of the skew sigmoid characteristic of arithmetic plots to a straight line in the middle response range. An added advantage is that such plots often differ little from log-probability plots in the middle response range, thus permitting more confidence in the reproducibility of the result. (When the plot is made directly on semi-logarithmic paper, and the herbicide concentrations are selected to give equal intervals on the X-axis, the line of best fit calculated by the least squares method can be found rapidly by graphic methods (Sher, 20). This should be attempted, of course, only when there is no obvious systematic deviation from linearity.) And finally, there are theoretical reasons (Westerfeld, 23; Bliss, 2) supported by experimental evidence in biological responses, for preferring a plot of log-concentration vs. response over an arithmetic plot.

Another technique which successfully relates current concepts in biochemistry to experimental results, and which is also in some danger of misconstruction, is the double-reciprocal plot.

THE DOUBLE-RECIPROCAL METHOD

The general scheme was developed in connection with enzyme activity by Lineweaver and Burk (16). J. Bonner and his group at the California Institute of Technology have extended the concepts from enzymes to auxin activity successfully. Epstein and Hagen (10) have used the device in fruitful investigations of cation penetration into roots. And there are several papers by Frans and associates (11) attempting to apply the procedure to herbicide research.

The formulation of Bonner's group (McRae et al., 17) proposed that where "E" represents a receptive enzymatic receptor site and "S" represents some auxin substrate, S and E combine reversibly and may react to give the irreversible response, Growth, thus:

$$E + S \underset{}{\overset{K_s}{\rightleftharpoons}} ES \xrightarrow{k} \text{GROWTH}$$

The net constant for the reversible reaction is "K_m", and for the irreversible reaction is "k". Frans applied this directly to herbicides in the formulation

$$H + M \underset{}{\overset{K_m}{\rightleftharpoons}} HM \xrightarrow{k} \text{INHIBITION OF GROWTH,}$$

where "H" was the herbicide concentration which combined with some vital mechanism "M". Presumably, when all available M is combined with H, the concentration HM is at maximum, and inhibition of growth is at maximum. Then, said Frans, "v", the initial velocity of the inhibition, is

$$v = \frac{V_{max}\,(H)}{K_m + (H)}$$ where K_m is the equilibrium

constant of the reversible reaction, and V_{max} is the constant representing the maximum velocity of the growth inhibition when all available receptor sites (or mechanisms) are saturated with herbicide. This can be rewritten in the linear form $y = b + mx$ by transforming to

$$\frac{1}{v} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}} \cdot \frac{1}{(H)},$$ and when $\frac{1}{v}$ is plotted against $\frac{1}{(H)}$

Frans' data with respect to several herbicides provided a statistically satisfactory linear relationship. Now the Y-intercept in such plots is considered a measure of $\frac{1}{V_{max}}$, and K_m, which is thought (erroneously —see below) to be determined from the slope $\frac{K_m}{V_{max}}$, is taken as a measure of the affinity of herbicide H for receptor site M, and is also characteristic of the system. The Y-intercept may show whether different herbicides are reacting at the same receptor sites—i.e. have similar modes of toxicity. Although equal Y-intercept values do not constitute proof, dissimilar values constitute negative evidence. This follows from the work of McRae et al. (17) with auxin inhibitors. They showed that when auxins and potential inhibitors of the response were allowed to act together in their system, they could distinguish between competitive, non-competitive, and an "uncompetitive" inhibition (Figure 5). Friedenwald and Maengwyn-Davies (12) derived similar figures based on theoretical activity of dissociable or coupling inhibitors, with particular reference to enzyme kinetics. Epstein and Hagen (10) studied antagonism of cations and furnished experimental evidence for the diagrams of Figure 5, which appear well enough established for study of a single enzyme system.

Note that similar Y-intercepts occur when two materials are competing for the same receptor site. Frans expressed his results on inhibition of growth as a percent of the controls, and it will be apparent that whether herbicides act by the same or by different mechanisms, the plots will all lead to the same Y-intercept of 0.01, the reciprocal of 100—for 100% inhibition of growth at a high

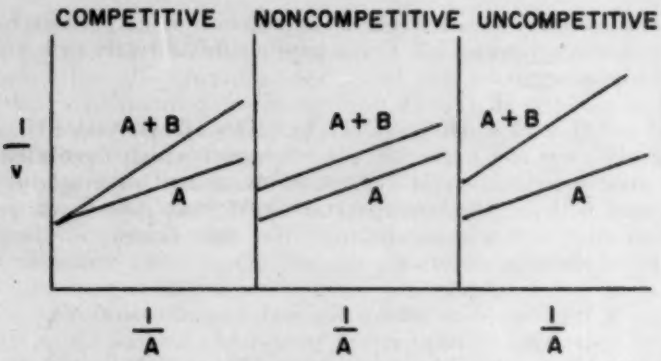

Figure 5. Theoretical results of plotting the reciprocal of initial reaction rate (v) vs. reciprocal of substrate concentration (A), showing the effect of an inhibitor (B), with three modes of antagonistic activity.

enough concentration. (See Figures 1–3, Frans et al. 11.) The evidence is only permissive, not definitive, in any case, but the conclusion may then be reached in experiments with herbicide mixtures that the materials may all be competitive in action. The false conclusion will be drawn, since the data lead to a common Y-intercept, that the materials are *not* non-competitive or uncompetitive. Synergism can not be detected, although antagonism, which may lead to less than 100% inhibition of growth, may be detectable.

For instance, data on the effects of combinations of herbicides applied by foliar dip (see Gowing, 13) are shown in Figure 6. The "expected" response line was plotted (on the basis of added probit mortalities) in search of synergism. No synergism in these mixtures was found. Field observations in tests of these chemicals show unmistakably that monuron does *not* kill by any mechanism competitive with sodium pentachlorophenate (PCP) or ethyl bis (dibutylamido) phosphate (EBP) despite the plots of Figure 6, which indicate that the chemicals *may* act competitively and do not act non-competitively or uncompetitively. However, some antagonisms, in which less than 100% toxicity is obtained, may be recognized (e.g. EBP and PCP; in this case probably owing to failure of these acutely toxic materials to translocate to the growing points of the plants). The double-reciprocal plot is an apparatus for investigating rate-limiting characteristics of the substrate (herbicide); fresh weight at harvest as a percent of the controls, which was the response measured to determine extent of toxicity, may be more limited by the *amount* of the herbicide rather than the rate of its availability at the receptor site. (The harvest was made 8 days after application of chemicals.)

A second point is that kinetic data do *not* provide a measure of the affinity of the substrate for the receptor site, despite the frequency with which the opposite view is asserted (e.g. Hofstee, 14). This has been thoroughly explained most recently by Reiner (18, p. 34), and derives from the circumstance that experimental data

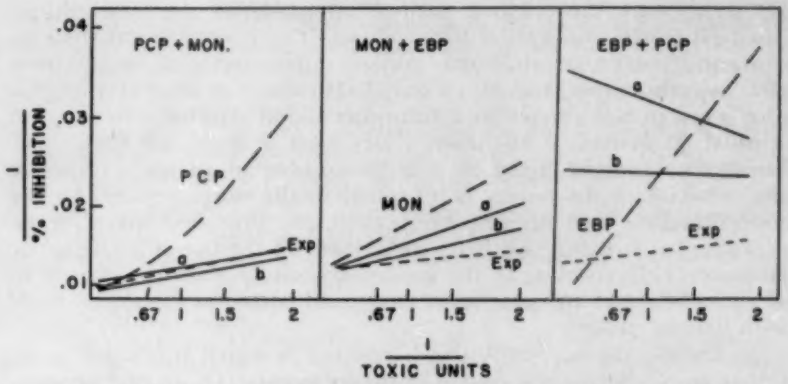

Figure 6. Double reciprocal plot of inhibition of growth of sunflower seedlings vs. concentration of mixtures of herbicides. Base chemical at range of toxic units, second chemical at (a) 0.44 or (b) 0.67 toxic unit. Exp = expected response on the basis of added mortalities. One toxic unit for Monuron—75 ppm; EBP —750 ppm; PCP—1200 ppm. This handling of these data is not justified—see text.

furnish information on K, the overall reaction rate constant, and not K_m. K appears to have no obvious interpretation in terms of affinity of substrate for the receptor, whether an enzyme or some other mechanism.

Three additional disquieting points may be examined briefly. First, a tactical error in the auxin work with double-reciprocal plots, noted by Bennett-Clark (1), is that v in the $\frac{1}{v}$ plot is initial velocity, and the *initial rate* of growth (or of inhibition in herbicide work) must be used for the plot. Frans and co-workers stated that the growth rate of their soybeans was linear over the period of 10 to 14 days, and this justified their use of fresh weight at harvest (usually a measure of final rate of inhibition rather than of initial rate). Other workers would do well to establish this linearity for their data or else make provision for plotting growth with time during the experiment and plotting back to "time zero" to find the initial rate. The acceptance of steady-state conditions in biological work as an indication of the initial conditions is common, but ignores the importance of the time factor, which, as suggested above, leads to confounding of the primary reaction with secondary results.

Next, it would be well to choose concentrations to provide for equal distribution of plotted points along the X-axis of the reciprocal plot. When points are "bunched" on a regression line, the points farthest from the main grouping have a disproportionate product moment, and this can lead to a speciously high regression coefficient when none is truly justified (Hofstee, 14; Eadie, 9).

Finally, it is a matter of some concern to the writer that the attractiveness of the "explanations" of herbicidal action in molecu-

lar terms may far outstrip proper attention to the assumptions involved in the conceptual formulation. The experimental data on concentration vs. response may indeed satisfactorily fit an equation of a hyperbola over the range tested. However, it is clear that this plot is not in fact a hyperbola, but must end at zero for zero toxicant applied. Moreover, it has many times been pointed out that curvilinear data may be fitted by a large number of curves, so long as the behavior of the curves is restricted to the range covered by the concentrations used (see also Westerfeld, 23). Provided only that the procedure is fruitful, this is no indictment of the use of such curves. However, inferences as to the modes of activity from the chance fit of a set of data to any particular curve and equation should be made with utmost caution.

As always, the acceptance of a concept is much increased by its utility in predicting the course of future events. Those investigating the mechanisms of herbicide toxicity would of course welcome some unifying theory on the nature of herbicide activity, even for a restricted group of compounds. Consequently, it is to be hoped that yet more rigorous examinations into the kinetics of inhibition of growth by herbicides will be continued.

SUMMARY

One of the important areas of current herbicide research is the testing of herbicide mixtures. Several methods of handling data in such tests are examined and critically discussed. Comparisons of percent response with concentration of herbicide, of response with log concentration, and of the reciprocals of percent response and concentration are considered. Certain cautions must be observed with any of the methods, although all may be useful.

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Effect of Histidine on the Inhibitory Action of 3–Amino–1,2,4–triazole

J. L. Hilton[1]

The present experiments were part of a study to determine the mechanisms by which herbicides affect plant growth and to find metabolites which protect growth against the inhibitory actions of herbicides. Bakers' yeast was selected for use in preliminary investigations because its rapid growth rate facilitates examination of large numbers of herbicide-metabolite combinations. The experiments reported herein present evidence which implicates histidine metabolism as a growth process which is sensitive to 3–amino–1,2,4–triazole [amitrole].[2]

Materials and Methods

Saccharomyces cerevisiae was isolated from the center of a cake of bakers' yeast (Fleischmann's). *Schizosaccharomyces pombe,* ATCC 4133, was obtained from the American Type Culture Collection. Histidine-requiring mutant strains of *Schizosaccharomyces pombe* (5), wild and mutant strains of *Salmonella typhimurium* (2) and the sample of histidinol were provided by Bruce N. Ames of the National Institutes of Health, Bethesda, Maryland.

Yeast cultures *(Saccharomyces cerevisiae* and *Schizosaccharomyces pombe)* were maintained on agar slants and subcultured in liquid medium as reported previously (3). Yeast growth experiments were carried out under aseptic conditions in 18 × 150-mm Pyrex test tubes. Each tube contained 1 ml each of herbicide and metabolite solutions and 6 ml of the pH 4.8 growth medium of King and Cheldelin (4) to which 200 μg/l of calicum pantothenate was added in lieu of β–alanine. After a 17-hour incubation at 32° C, growth was determined turbidimetrically with a Klett-Summerson colorimeter with a Number 42 filter. Control cultures generally gave turbidity readings of 140 to 160 units when compared to a nutrient solution blank. Mutant cultures were slower growing and were generally incubated for 42 hours. Experiments were run in triplicate with at least three different subcultures of the organisms.

Salmonella typhimurium was cultured with aeration at 37° C in 250-ml Erlenmeyer flasks containing 50 ml of the pH 7 medium of Vogel and Bonner (6). Growth was determined turbidimetrically after 8 and 24 hours.

Results

The concentration of amitrole required for 50-percent inhibition of *Saccharomyces cerevisiae* growth was 3 to 4 × 10^{-5}M. Amitrole concentrations above 7 × 10^{-5}M appeared to cause almost complete inhibition of measurable growth during a 17-hour period. However,

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[2]The experimental sample of pure 3–amino–1,2,4–triazole was supplied by research personnel of Amchem Products, Inc., Ambler, Pennsylvania.

when the growth period was increased to 40 hours, the inhibitions caused by 10^{-4}, 5×10^{-4} and 10^{-3}M amitrole were reduced from 100 percent to 22, 75, and 93 percent, respectively. The concentration of amitrole required for inhibition during the shorter growth period depended partly upon the concentration of inorganic salts in the medium. Addition of 2×10^{-1}M NaCl or other salts to the medium resulted in a two- to three-fold increase in the amount of amitrole required for 50-percent inhibition. Inorganic salts did not reduce the toxicity of amitrole concentrations above 2×10^{-4} M. Salt concentrations as low as 10^{-3} did not alter amitrole toxicity.

Addition of 10^{-2}M *L*–histidine to the growth medium caused a 3000-fold increase in the amount of amitrole required to inhibit growth of *Saccharomyces cerevisiae* by 50 percent (Figure 1). Histidine alone had no effect on yeast growth; but the protective effect of *L*–histidine against amitrole toxicity was apparent at histidine concentrations as low as 10^{-6}M; and 10^{-4}M was nearly sufficient to produce the maximum effect obtained with this amino acid. Only *L*–histidine caused such a striking degree of reversal of the herbicide action as shown in Figure 1. No reversal of amitrole inhibition could

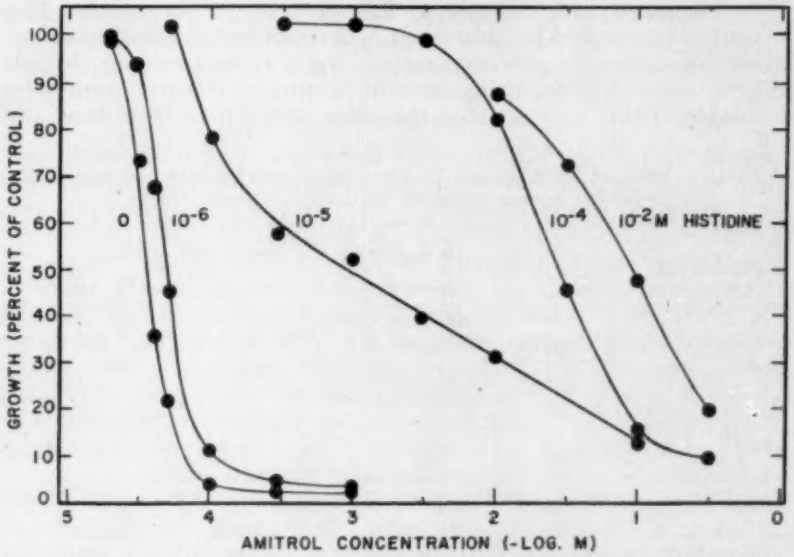

Figure 1. Effects of 0, 10^{-6}, 10^{-5}, 10^{-4}, and 10^{-2}M *L*–histidine in preventing inhibition of *Saccharomyces cerevisiae* growth by 3–amino–1,2,4–triazole (amitrole).

be obtained with *D*–histidine. Neither *L*–histidinol (the metabolic precursor of histidine) nor urocanic acid (the deamination product of histidine) circumvented the effect of amitrol.

The pronounced effect obtained with *L*–histidine could not be duplicated with 10^{-3}M concentrations of other *L*–amino acids or with purines or pyrimidines. However, some of these metabolites did reverse the inhibitory action of amitrole concentrations of 2×10^{-4}M

or lower. In order of decreasing effectiveness, the *L*–amino acids capable of partial reversal of amitrole inhibitions were leucine, tyrosine, methionine, isoleucine, tryptophane and possibly phenylalanine. Other common amino acids were ineffective. Glutamic acid failed to antagonize amitrole even when used as the sole source of nitrogen for growth. Six purines and pyrimidines investigated caused at least slight reversal of the amitrole inhibition. These compounds were of special interest since Aldrich reported indications of antagonism of amitrole by adenine and uracil in experiments with higher plants (1). In order of decreasing effectiveness in the yeast experiments, the compounds partially reversing amitrole action were cytosine, guanine, adenine, hypoxanthine, 4–amino–5–imidazole carboxamide and uracil. The ribose nucleosides of these metabolites were less effective than the free bases. Next to *L*–histidine the most effective compounds examined were cytosine and *L*–leucine. These two metabolites accomplished a small reduction in amitrole toxicity but failed to reverse the action of amitrole concentrations above 2×10^{-4}M. Histidine, however, partially reversed the action of amitrole concentrations as high as 3×10^{-1}M.

The ability of *L*–histidine to circumvent the action of amitrole was then examined with cultures of *Schizosaccharomyces pombe*. This organism was chosen because (a) growth could be obtained under the same conditions used with *Saccharomyces cerevisiae* and (b) five different histidine-requiring mutant strains of the organism were available. Table 1 shows that the effect of amitrole, histidine, and

Table 1. Effect of amitrole and histidine on growth of wild and histidine-requiring mutant strains of *Schizosaccharomyces pombe*.

Histidine conc.	Growth (turbidity) at amitrole conc. of				
	0	10^{-4}M	5×10^{-4}M	5×10^{-3}M	10^{-2}M
Wild strain *S. pombe*[a]					
0	148	25	—	—	30
10^{-4}M	142	141	—	—	126
Mutant strain *S. pombe*[b]					
0	0	—	—	—	—
10^{-5}M	58	53	55	50	54
2×10^{-5}M	96	95	95	98	94
3×10^{-5}M	117	114	113	115	126
5×10^{-5}M	145	137	136	144	144
10^{-4}M	146	137	137	137	144

[a]17-hour growth period.
[b]42-hour growth period.

amitrole-histidine combinations on growth of the wild strain of the organism were similar to those observed with *Saccharomyces cerevisiae*. However, amitrole did not inhibit growth of the histidine-requiring mutant strain in the presence of histidine concentrations from 10^{-5} to 10^{-4}M (Table 1). The mutant organism grew more slowly than the wild strain; therefore, the effect of amitrole was observed after 20- and 42-hour incubation periods. Amitrole did not

inhibit growth during either period. The growth of all five mutant strains was observed in 10^{-5} and 10^{-4}M histidine in combination with 10^{-2} and 5×10^{-2}M amitrole. No antagonism was observed between these chemicals in their action on growth of the mutant organisms. Such evidence suggests that amitrole does not prevent the utilization of histidine by *Schizosaccharomyces pombe*.

Histidine antagonism of amitrol action was not observed with all micro-organisms. Under the conditions used in these experiments, growth of *Salmonella typhimurium* was relatively insensitive to the herbicide and failed to show inhibition by 10^{-3}M amitrole. At higher concentrations amitrole reduced the rate of growth but not the total growth. During an 8-hour growth period 10^{-2}M amitrole caused complete inhibition of growth, but no inhibition was apparent after 24 hours. This reduction in growth rate by 10^{-2}M amitrole was not reversed by histidine concentrations up to 10^{-4}M when either the wild strain or a histidine-requiring mutant strain of the organism was used.

Wheat, oats, barley and tomatoes were then used in experiments to determine whether histidine could circumvent the action of amitrole in higher plants. All investigations were performed with intact seedlings. A variety of techniques were used for application of the two chemicals to seedlings. No evidence was obtained to demonstrate antagonism between histidine and amitrole in intact plants.

DISCUSSION

Evidence for a protective effect by *L*–histidine against the growth inhibitory action of amitrole has been observed in experiments with *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. The amount of amitrole required to cause 50-percent inhibition of *Saccharomyces cerevisiae* growth was increased from 4×10^{-5}M to 10^{-1}M by the addition of *L*–histidine to the nutrient medium. A comparable effect could not be obtained with *D*–histidine, *L*–histidinol,[3] urocanic acid, *L*–amino acids, purines or pyrimidines.

The amitrole–histidine interactions shown in Figure 1 could result from direct competition between the two chemicals for positions in or on the yeast cells; but the experiments with the yeast mutants do not support this interpretation. If amitrole and histidine are competitive, one would expect amitrole inhibition of growth of the histidine-requiring mutants to be inversely proportional to histidine concentration. However, amitrole did not inhibit growth of the mutants. Therefore, the hypothesis that amitrole competes directly with histidine at either the cell membrane level (permeability) or at the enzyme level (metabolic utilization) seems unlikely.

The effect of *L*–histidine is probably best explained by the hy-

[3]Histidinol is not taken up by Neurospora and is only slowly taken up by bacteria (Bruce N. Ames, personal communication). It did not support growth of the histidine-requiring mutant strains of *Schizosaccharomyces pombe* used in the present study. Therefore, the inability of histidinol to prevent inhibition of growth by amitrole may be due to impermeability of the yeast cells to the metabolite.

pothesis that histidine represents the product of some metabolic pathway which can be inhibited by amitrole or by a metabolized form of amitrole. Since this pathway is genetically blocked in the histidine-requiring mutants, the failure of amitrole to inhibit their growth is expected. Additional investigations will be necessary to confirm this hypothesis and to establish the exact nature of the histidine–amitrole interactions.

The protective effect of histidine against amitrole toxicity has been demonstrated with two species of yeast; but a similar effect was not observed with one species of bacteria or four species of higher plants. These negative results cast considerable doubt on the importance of histidine metabolism in the toxic action of amitrole in most organisms. However, the protective effect of histidine may be hidden in these organisms by amitrole inhibition of other equally sensitive growth processes or by physical barriers blocking absorption, penetration or translocation of the metabolite. Therefore, as a working hypothesis for future investigations, histidine metabolism should not be overlooked as a possible process through which amitrol alters growth or chemical quality of plants.[4]

SUMMARY

Inhibition of growth of *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* by amitrole has been observed. An exogenous supply of *L*–histidine has been shown to produce varying degrees of protection against these inhibitions. Amitrole did not inhibit growth of histidine-requiring mutant strains of *Schizosaccharomyces pombe*. Evidence for antagonism between histidine and amitrole in their effects on growth of one species of bacteria and four species of higher plants was not demonstrated by the techniques used in these studies.

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Field Bindweed Control by Combinations of Cropping, Cultivation, and 2,4–D[1]

O. G. Russ and L. E. Anderson[2]

Investigations during the past three decades have confirmed that field bindweed *(Convolvulus arvensis)* is exceedingly difficult to eradicate. Since the introduction of sodium chlorate by Latshaw and Zahnley (1) various other soil sterilants have been used successfully for spot treatments. Timmons (3) reported the effect of various systems of intensive cultivation on bindweed control as influenced by root reserves. Phillips and Timmons (2) reported the response of bindweed to crop competition and combinations of cropping, cultivation and 2,4–D. The introduction of 2,4–D (2,4–dichlorophenoxyacetic acid) provided a new tool for bindweed control and an impetus for continued research.

Experiments described in this paper were begun in 1947 at the Bindweed Experiment Field, Canton, Kansas, to obtain information on the effectiveness of 2,4–D in combination with cultivation and various cropping systems.

Materials and Methods

The Canton Bindweed Experiment Field includes 80 acres of level land with a dense, uniform stand of bindweed. The soil, which is not uniform, has been classified as a clay-loam surface with a slowly permeable clay subsoil. Drainage is a problem during periods of excessive rainfall.

Plots 1×4 rods were first laid out the spring of 1947. Field equipment was used for all tillage operations; cultivations were made with a duckfoot cultivator having 16-inch sweeps. In 1947 all 2,4–D applications were made at a rate of 2 lb/A. In 1948 and thereafter 1½ lb/A was applied on uncropped plots and 1 lb was used where crops were grown.

A split plot comparison of the butoxyethanol ester and the diethylamine salt of 2,4–D was made from 1947 through 1951. Since no difference in effectiveness was observed during that period, only the butoxyethanol ester was subsequently applied. Duplicate plots were used in all experiments.

The various combinations of cropping, tillage, and 2,4–D applications included were grouped into five categories. Numerous variations were introduced under each of these general systems. Each system was scheduled to be continued over the period of time required to obtain satisfactory control. In general, 2,4–D was applied when bindweed was in good vegetative growth. The maximum

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length of time included in the study was six years. Following are the general methods, including variations:

I. Continuous wheat (sowing date: October 7).
   A. Spray wheat when bindweed growth is adequate in spring; plow after harvest; cultivate prior to sowing.
   B. Spray wheat when bindweed growth is adequate in spring; plow after harvest; spray August 15–31; cultivate prior to sowing.
   C. Plow after harvest; spray August 15–31; cultivate prior to sowing.
   D. Plow after harvest; cultivate to September 1; spray prior to sowing.

II. Continuous oats.
   A. Spray bindweed in fall; sow oats in spring; spray bindweed in spring when growth is adequate; cultivate between harvest and September 1.
   B. Spray bindweed in fall; sow oats in spring; cultivate between harvest and September 1.

III. Continuous forage sorghum (drilled).
   A. Spray bindweed in spring prior to disking, cultivation and seedbed preparation.
   B. Spray in fall; cultivation as necessary in spring prior to seedbed preparation.
   C. Spray in fall; cutivate as necessary in spring prior to seedbed preparation; plant sorghum; spray when bindweed growth is adequate.

IV. Continuous forage sorghum (listed with moldboard lister).
   A. Spray bindweed in spring when growth is adequate; prepare seedbed; list sorghum (40 inch rows); cultivate as necessary.
   B. Cultivate in spring; prepare seedbed; list sorghum (40 inch rows); spray bindweed when growth is adequate; cultivate as necessary.

V. Cultivation (no crop).
   A. Spray in spring when bindweed growth is adequate; cultivate as needed until frost.
   B. One cultivation in spring; spray when bindweed growth is adequate; cultivate as needed until frost.
   C. Cultivate as needed until July 1; spray when growth is adequate; cultivate as needed until frost.
   D. Cultivate as needed until September 1; spray when growth is adequate; cultivate as needed until frost.

Treatments were evaluated by making plant counts in random 4-foot square quadrats. Evaluations were expressed in percentage of bindweed control compared to untreated checks. Final evaluations of the various treatments were made during the fall of the year as indicated in Table 1.

Table 1. Summary of experimental results using 2,4-D in combination with cultivation and cropping systems to control field bindweed.

	Year begun	Year concluded	Av. cultivations per year	No. 2,4-D app'l per year	Av. crop yield	Av. yield on bindweed free plots	Percent bindweed control
I. Continuous wheat.							
A. Spray wheat in spring; plow after harvest; cultivate as necessary prior to sowing	1948	1953	4.3	1	*Bu/A* 17.0	*Bu/A* 28.0	93
B. Spray wheat in spring; plow after harvest; spray Aug. 15–31; cultivate as necessary prior to sowing	1948	1953	5.0	2	15.5	28.0	95
C. Plow after harvest; spray Aug. 15–31; cultivate as necessary prior to sowing	1947	1952	4.5	1	15.3	29.9	97
D. Plow after harvest; cultivate to Sept. 1; spray prior to sowing	1947	1952	3.5	1	19.3	29.9	95
II. Continuous oats.							
A. Spray bindweed in fall; sow oats in spring; spray bindweed in spring when growth is adequate; cultivate between harvest and Sept. 1	1947	1952	3.0	2	18.9	30.0	88
B. Spray bindweed in fall; sow oats in spring; cultivate between harvest and Sept. 1	1948	1953	3.6	1	23.6	27.0	80
III. Continuous forage sorghum (drilled).					*Tons/A*	*Tons/A*	
A. Spray bindweed in spring prior to cultivation and seedbed preparation	1947	1950	2.5	1	4.7	10.5	90
B. Spray in fall; cultivate as necessary in spring prior to seedbed preparation	1947	1950	3.6	1	4.9	10.5	90
C. Spray in fall; prepare seedbed in spring; plant sorghum; spray when bindweed growth is adequate	1947	1950	3.7	2	4.6	10.5	90
IV. Continuous forage sorghum (listed).							
A. Spray bindweed in spring when growth is adequate; prepare seedbed; list sorghum (40-inch rows); cultivate as necessary	1949	1954	6.1	1	7.2	9.8	96
B. Cultivate in spring; prepare seedbed; list sorghum (40-inch rows); spray bindweed when growth is adequate; cultivate as necessary	1949	1954	7.5	1	3.2	9.8	96
V. Cultivation (no crop).							
A. Spray in spring when growth is adequate; cultivate as needed until frost	1948	1950	5.4	1	—	—	100
B. One cultivation in spring; spray when growth is adequate; cultivate as needed until frost	1948	1950	6.0	1	—	—	100
C. Cultivate as needed until July 1; spray when growth is adequate; cultivate as needed until frost	1948	1950	5.6	1	—	—	98
D. Cultivate as needed until Sept. 1; spray when growth is adequate; cultivate as needed until frost	1948	1950	7.3	1	—	—	100

RESULTS AND DISCUSSION

Continuous wheat.

All treatments under this system were effective in bringing heavy infestations of field bindweed under control. In making spring applications of 2,4–D to bindweed in wheat, it was found that 2,4–D injury reduced wheat yield. A spring application of 2,4–D may be justified by prevention of bindweed seed production, reduction in stand, and facilitation of harvest. Two applications of 2,4–D per year were no more effective than one.

In comparing cultivation with an application of 2,4–D between harvest and sowing, slightly better results were obtained by using 2,4–D. This suggests the use of cultivation only during seasons adverse for bindweed growth and 2,4–D when conditions are favorable for a good stand.

Early plowing to permit maximum bindweed growth by late summer followed by an application of 2,4–D resulted in good control. This method of treating bindweed reduced the hazard of injury which often occurred when using 2,4–D on a growing wheat crop.

Continuous spring oats.

Fall and spring applications of 2,4–D in continuous oats resulted in better bindweed control than spring applications alone but was accompanied by a reduction in yield. Continuous oats did not control bindweed as well as continuous wheat. Furthermore, oats are not as well adapted to Kansas climatic conditions as wheat, and land devoted to oats production is more subject to wind erosion during the winter and early spring.

Continuous forage sorghum.

In the continuous forage sorghum system, Atlas provided vigorous competition during the growing season. In comparing the use of 2,4–D in close drilled and listed Atlas sorghum the listed gave slightly better control. This difference can be attributed in part to better coverage when bindweed was sprayed between the rows in listed sorghum.

Intensive cultivation ± 2,4–D.

Under the conditions that existed at the Canton Bindweed Field, cultivation with a duckfoot cultivator at 2–3 week intervals throughout the growing season was the most effective method used to control bindweed. Time of 2,4–D application during the growing season did not appear to result in any great difference in degree or rapidity of control. It will be noted in Table 1 that these treatments extended over a three-year period while all other systems were evaluated after a period of six years. In evaluating the worth of an intensive cultivation system, the economics involved with respect to loss of crops for the three-year period must be considered.

SUMMARY

Various combinations of cropping, cultivation, and 2,4–D applications were evaluated for control of field bindweed. These systems

with their numerous variations can be grouped as follows: (1) continuous winter wheat, (2) continuous spring oats, (3) continuous drilled forage sorghum, (4) continuous listed forage sorghum, and (5) intensive cultivation.

Based on the conditions that existed during the course of this experiment a three-year system of intensive cultivation in combination with an annual application of 2,4–D resulted in most effective bindweed control. A combination of listed forage sorghum, cultivation, and 2,4–D over a six-year period was slightly superior to a combination of wheat, cultivation and 2,4–D. Crop yields were reduced by all systems employed in attempting to control bindweed.

No single method of control was found to be acceptable in all situations. It was evident that under existing conditions complete bindweed eradication on a field scale may not be realized or it may be impractical from an economic standpoint. This suggests the adoption of control methods that suppress rather than eradicate bindweed.

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Characteristics of Johnsongrass Rhizomes[1]

L. E. Anderson, A. P. Appleby, and J. W. Weseloh[2]

Introduction

Rhizome initiation and growth is a contributing factor to the problem of controlling Johnsongrass (Sorgum halepense (L.), Pers.). Control methods by intensive cultivation or chemical applications are directed primarily toward the destruction of these underground structures. As described by Fletchall et al. (3) rhizomes are fleshy, segmented underground stems having scales at the nodes. Their development is rapid; they function as organs for carbohydrate storage, and as reproductive structures which produce vegetative shoots from axillary and terminal buds. Information relative to development of rhizomes and their response to various treatments is reported; this is essential to understanding and evaluating methods of control.

Materials and Methods

Investigations concerned with early development of rhizomes were conducted in the greenhouse while plants which grew to the seed maturity or winter dormancy stages were grown outdoors. Single plants were established in pots or tubs from germinating seeds thinned to one plant per container. All plants were grown without competition in fertile, well-watered soil. Greenhouse temperature fluctuated between 78–84° F. Plants were removed from the pots at weekly intervals, and observed for rhizome initiation and development.

Longevity was determined by marking rhizome sections with plastic coated wire loops and burying individual sections in separate tubs of soil. When the resulting topgrowth had matured seed, the marked rhizome sections were recovered by washing away the soil and removing all root and new rhizome growth. The original marked sections were reburied in soil and observed for shoot development or for decay and decomposition.

The reproductive potential of rhizomes was determined by placing uniform sections consisting of eight internodes in moist vermiculite. Rhizome sections were taken from the vermiculite at weekly intervals; vegetative shoots removed and the sections replaced in the vermiculite. This procedure was continued for a ten-week period when the rhizomes were exhausted. Treatments included intact rhizome sections; sections broken to individual nodes, and sections segmented to nodes after vegetative shoots had been removed through a five-week period.

Influence of moisture content on rhizome viability was determined

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by air drying to various moisture percentages based on initial weight. Moisture percentage was started by drying to 50% of the initial weight and then at successive decrements of 5% to a low of 20%... the minimum weight percentage level reached by air drying. After drying to specified percentages, the rhizome sections were placed in moist vermiculite to determine recovery based on their potential to produce vegetative shoots.

Rhizome sections used in the various experiments were obtained in the spring from established stands of Johnsongrass located in Riley County, Kansas. Only sections of uniform size and weight were used. Considerable variation in rhizome diameter and length of internodes was observed. Bennett (1) has reported variation in Johnsongrass culms, panicles and seed production. Three rhizome sections were included in each plot in all experiments; all treatments were replicated three times.

RESULTS AND DISCUSSION

Early rhizome development in a seedling is illustrated in Figure 1. This stage of growth was characteristic of seedlings from six to

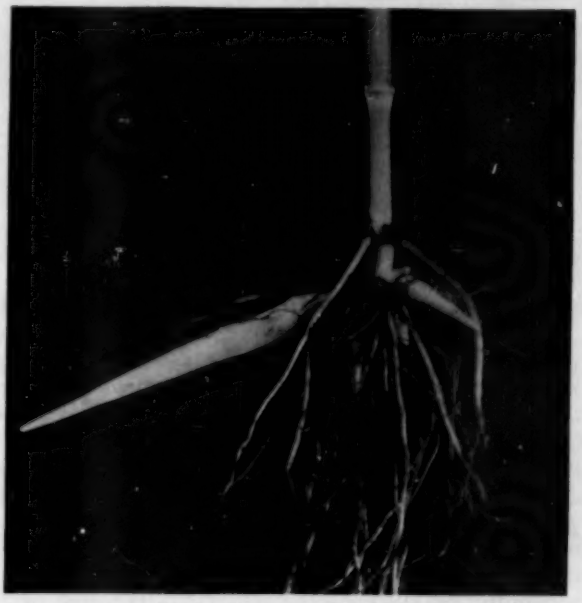

Figure 1. Root and rhizome development of a Johnsongrass seedling six weeks after emergence.

seven weeks after emergence. Rhizome initiation occurred approximately one week earlier. A rapid acceleration in development was observed from the rhizome initiation stage through the matured-seed stage of growth. A similar pattern of rhizome development has been reported by Oyer et al. (5). A rhizome system recovered at the

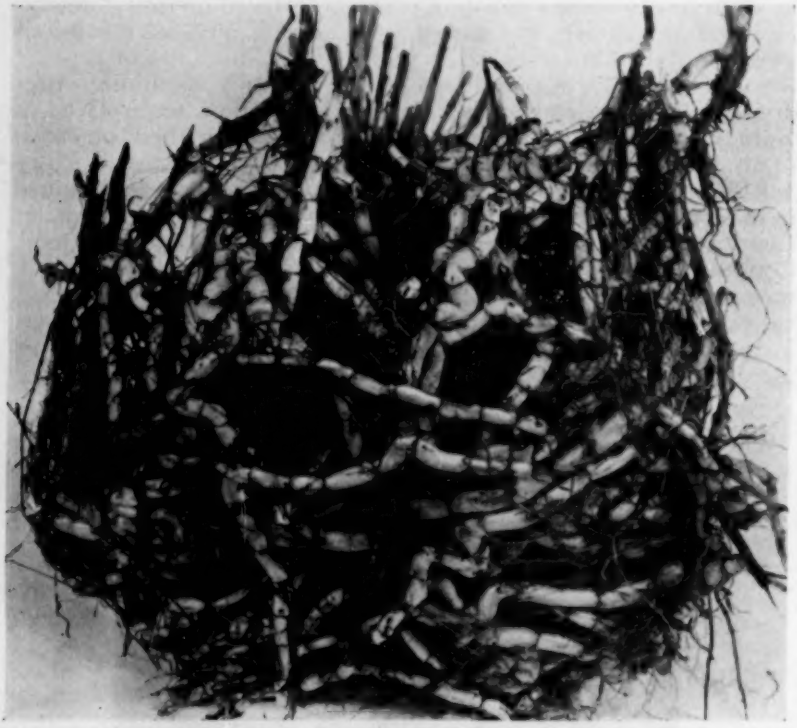

Figure 2. Root and rhizome development of a 4½ months old Johnsongrass plant at the winter dormancy stage. Rhizome internodes numbered more than 5200.

winter dormancy stage is illustrated in Figure 2. Under the conditions of this experiment it was found that greatest increase in rhizome development occurred from the mature seed stage to the winter dormancy stage. During this period rhizomes function primarily as storage organs and carbohydrate buildup is rapid. Consequently fall plowing or cultivation following the mature seed stage to inhibit rhizome growth and associated food storage would be essential in a control program. However, Burt and Willard (2) report July 30 plowing to be most effective in controlling Johnsongrass under Ohio conditions.

Marked rhizome sections obtained in the spring and buried in soil in the greenhouse produced vigorous vegetative and underground growth. Upon being detached at the mature seed stage the recovered rhizome sections were dark in color, slightly shrunken and woody, but appeared firm and sound. When buried in the soil a second time new shoots failed to develop and the rhizomes gradually decayed. This observation indicates that rhizomes which develop during a given growing season overwinter and give rise to new plants the following year, but are unable to function longer. Talbot (6) reported similar observations.

In determining reproductive potential of rhizomes, no significant difference was found in the number of vegetative shoots produced under the various treatments. From a total of 12 intact rhizome sections 87 shoots were removed during the ten-week period; segmented sections produced 83 shoots; and intact sections later segmented produced 80 shoots. Rhizome section means for treatments were 7.25, 6.92 and 6.67 respectively. Although not significantly different, the trend appears to indicate that unbroken sections produced more shoots than rhizomes broken into segments.

The influence of rhizome drying was expressed by a sharp reduction in vegetative shoots produced below a level of 40% of the original weight. Shoots developed in all replications at levels of 45% and 40% drying but appeared only in scattered rhizome sections at the 35, 30 and 25 percentages. When rhizomes were dried to 20% of their original weight, they were injured beyond the point of recovery and did not produce a single vegetative shoot. According to Overpeck (4), rhizomes can be killed by tillage practices that leave them on the soil surface where exposure to sun and wind will dry them beyond the point of recovery. On the basis of evidence presented, it appears that this control method would be ineffective except where rhizome exposure is accompanied by maximum drying.

SUMMARY

Characteristics of Johnsongrass rhizomes reported include initiation and development, longevity, reproductive potential, and viability under various moisture levels.

It was found that initiation of rhizomes in seedlings occurs approximately four to five weeks following emergence. Well developed rhizomes were evident after a six- to seven-week period. Rhizomes continued to develop at a rapid rate up to the winter dormancy stage.

Marked rhizome sections buried in soil produced plants and new rhizomes but were unable to propagate new growth when buried a second time.

Uniform rhizome sections placed in moist vermiculite produced most vegetative shoots when left intact and least shoots when broken into segments after a five-week period. Sections broken into individual segments were intermediate in shoot development, but the differences were not significant.

Rhizomes remained highly viable when dried to 40% of their initial weight. Sporadic vegetative shoots were produced when dried to 25% but no shoots developed at the 20% level.

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Chemistry of Some Amino Acid Derivatives of 2,2–Dichloropropionic and 2,3,6–Trichlorobenzoic Acids

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THIS report describes the preparation, purification, and properties of 18 new herbicides, amino acid derivatives of 2,2–dichloropropionic acid (dalapon) and 2,3,6–trichlorobenzoic acid (2,3,6–TBA). The biological evaluation of the compounds as selective herbicides is being made by other investigators and will be published later. Some of the compounds are also being evaluated for nematocidal, fungicidal, and anticancer properties.

Previous experience with a variety of chlorine substituted phenoxy acids has demonstrated that amino acid attachment through amide linkage can substantially affect the selective herbicidal and growth-regulating properties of phenoxy compounds[2, 3] (2, 3, 4, 5, 6, 8). In these studies it was found that plant growth regulation depended, not only upon the amino acid and phenoxy acid coupled, but it also depended upon the optical configuration of the compounds prepared; hence, the reasons for preparing these two series with the use of several amino acids in their *D-*, *L-*, and *DL*-configurative forms.

A. AMINO ACID DALAPON COMPOUNDS

As a herbicide, dalapon is generally more toxic to monocotyledonous than to dicotyledonous plants. Because of this property, the compound is most widely used in the control of grasses in agronomic and horticultural crops. The limiting factor with respect to the merit of dalapon in its many applications is its phytotoxicity. Derivatives of dalapon having lower toxicity and greater selectivity would be desirable.

Of the series of derivatives reported here, only one compound, N–(2,2–dichloropropionyl)–*L*–leucine, has been evaluated for herbicidal properties so far.[4] In post-emergence spray applications responses of crop plants to this derivative were more pronounced in 11, less pronounced in 7 plants. In 7 weed species tested the amino acid derivative was more toxic to 4 species, less toxic to 1 species, than the parent dalapon.

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[2]Unpublished data. Shaw, W. C. and Gentner, W. A., Weed Control in Crops Section, Crops Research Division, ARS, USDA, Plant Industry Station, Beltsville, Md.

[3]Krewson, C. F., Carmichael, J. F., Drake, T. F., Mitchell, J. W., and Smale, B. C., "Growth regulators. Synthesis and preliminary evaluations of amino acid derivatives of *DL*–2–(2,4,5–trichlorophenoxy)–propionic acid."

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Synthesis of amino acid derivatives of dalapon.

In order to chemically unite an amino acid through an amide linkage to dalapon it was first necessary to convert the latter to its chloride, 2,2–dichloropropionyl chloride.

2,2–Dichloropropionyl chloride (I). This compound was prepared in 87.1% yield by the reaction of dalapon[5] (3.0 *M*) with thionyl chloride (4.0 *M*) by the method of Freed (1). The acid chloride distilled at 36–40°/0.6–0.8 mm., n_D^{25} 1.4540.

Anal. Calcd. for $C_3H_3Cl_3O$: Cl, 65.8% Found: Cl, 64.7%.

Either of the following two general procedures (2, 7) were used in the preparation of the amino acid derivatives. The Ronwin technique (7) was used only for the *D*– and *L*– threonine derivatives. The amino acids used were of the best quality obtainable from commercial sources.

N–(2,2–Dichloropropionyl)–D–leucine (II). To 13.1 g. (0.1 *M*) of *D*–leucine dissolved in 200 ml. (0.3 *M*) of 1.5 *N* sodium hydroxide solution was added dropwise a solution of 16.1 g. (0.1 *M*) of I dissolved in 150 ml. of benzene. The reaction mixture was chilled to 5° and stirred mechanically. Upon complete addition the ice bath was removed and the mixture stirred for an additional 3 hours as it warmed to room temperature. The reaction mixture was extracted with ethyl ether and the ether extracts combined and washed with 100 ml. of distilled water. The water wash was separated and added to the alkaline aqueous solution of the product which was then acidified to a pH 3 with 1 *N* hydrochloric acid. A white precipitate resulted during the addition of the hydrochloric acid. After cooling this mixture in the refrigerator for 2 hours, the precipitated product was extracted with ethyl ether. The ether solution was washed with water and dried over anhydrous calcium sulfate. The ether solution was filtered and the solvent removed using an air current. The vacuum dried crude material weighed 18.0 g (70.3%), m.p. 100–104° (Kofler micro melting point apparatus). Crystallization from hot ethyl acetate using petroleum ether (b.p. 63–70°) as a precipitating agent gave 14.0 g. (54.6%) of pure II, m.p. 104.0–105.0°.

Anal. Calcd. for $C_9H_{15}Cl_2NO_3$: Cl, 27.68%; N, 5.46%. Found: Cl, 27.24%; N, 5.53%.

N–(2,2–Dichloropropionyl)–D–threonine (III). To 10.6 g. (0.08 *M*) of *D*–threonine suspended in 200 ml. of ethyl acetate was added 12.9 g. (0.08 *M*) of I dissolved in 100 ml. of ethyl acetate. The reaction mixture was refluxed for 30 hours as it stirred mechanically. The warm reaction mixture was filtered and the solvent evaporated leaving an oil. Repeated slurring of the oil with petroleum ether gave a white solid. The vacuum dried crude material weighed 9.0 g. (46.1%), m.p. 115.0–120.0°. Crystallization from hot ethyl acetate-petroleum ether and from methylene chloride-petroleum ether gave 6.9 g. (35.3%) of pure III, m.p. 118.5–120.0°.

[5]Supplied through the courtesy of the Dow Chemical Company, Midland, Michigan. Mention of commercial products or names does not imply that they are endorsed or recommended by the Department of Agriculture over others of a similar nature not mentioned.

Anal. Calcd. for $C_7H_{11}Cl_2NO_4$: Cl, 29.5%; N, 5.73%. Found: Cl, 28.94%; N, 5.81%.

Table 1 contains data on these and the other amino acid derivatives of dalapon prepared.

Table 1. Yields, physical properties and analyses of amino acid derivatives of 2,2-dichloropropionic acid.

N-(2,2-dichloropropionyl)-	M.P. (corr.)[a]	Yield, %		Analyses[b]				
				Cl, %		N, %		Optical rotation[c]
		Crude	Refined	Calcd.	Found	Calcd.	Found	$[\alpha]_D^{25}$
L-leucine	104.0–106.0[d]	76.1	44.0	27.68	27.50	5.46	5.47	−6.33 ± 0.4
D-leucine	104.0–105.0	70.3	54.6	27.68	27.24	5.46	5.53	+7.03 ± 0.4
DL-leucine	85.0–86.0[e]	82.7	26.1	27.68	27.16	5.46	5.49	
L-methionine	55.0–56.5[f]	71.5	31.7	25.87	26.00	5.13	5.14	−3.37 ± 0.4
D-methionine	52.0–55.0	58.0	35.1	25.87	25.58	5.13	5.06	+2.19 ± 0.4
DL-methionine	56.0–58.0	35.5	25.9	25.87	25.66	5.13	5.07	
L-threonine	124.0–125.0	48.3	16.5	29.05	28.86	5.73	5.73	−14.2 ± 0.4
D-threonine	118.5–120.0[g]	46.1	35.3	29.05	28.94	5.73	5.81	+13.55 ± 0.3
DL-threonine	180.0–182.0[h]	—	17.5	29.05	29.45	5.73	5.69	

[a]Recrystallized one or more times from ethyl acetate-petroleum ether unless otherwise indicated.
[b]Analyses by J. S. Ard.
[c]Concentration 2.00 g./100 ml. in pyridine.
[d]As in a; also from benzene-petroleum ether.
[e]Recrystallized from petroleum ether only.
[f]As in a; also from petroleum ether.
[g]As in a; also from methylene chloride-petroleum ether.
[h]No recrystallization necessary.

B. Amino Acid Derivatives of 2,3,6-Trichlorobenzoic Acid

Recently Gentner and Shaw[6] reported on the comparative herbicidal activity and selectivity of 14 variously substituted benzoic acids or derivatives. All of these were tested on 26 crop plants and weed species as pre-emergence and 12 were tested as post-emergence sprays. A *L*-leucine derivative of 2,3,6-TBA, the only amino acid compound of this new series tested to date, reduced the activity of the highly active moiety of 2,3,6-TBA in both pre- and post-emergence applications. This suggested the possibility of tailor-making specificity and selectivity into the 2,3,6-TBA molecule with amino acids.

Synthesis of 2,3,6-trichlorobenzoic acid derivatives.

Schotten-Baumann procedures similar to those previously described (4, 8) were employed in the preparation of amino acid derivatives of 2,3,6-TBA. The 2,3,6-TBA was supplied as a solution of the dimethylamine salt[7] (4 lb/gal). To effect precipitation of the 2,3,6-TBA this solution was adjusted to pH 2 with 10% hydrochloric acid solution. The 2,3,6-TBA was then filtered off and recrystallized twice from hot toluene and once from hot petroleum ether (b.p. 63–70°); m.p. 2,3,6-TBA 135.5–138°.

[6]Gentner, W. A., and Shaw, W. C. *loc. cit.*[4]
[7]Supplied by R. W. Varner of E. I. duPont de Nemours and Company, Wilmington, Delaware.

2,3,6–Trichlorobenzoyl chloride. The purified 2,3,6–TBA was converted to its corresponding chloride by the method of Freed (1). A yield of 96% was obtained with a 0.5 *M* quantity of 2,3,6–TBA and 1.5 *M* thionyl chloride; boiling point of product 68–69° at 0.2 mm., n_D^{26} 1.5900.

Anal. Calcd. for $C_7H_2Cl_4O$: C, 34.46%; H, 0.83%; Cl, 58.15%. Found: C, 34.52%; H, 0.59%; Cl, 58.11%. Product has remained a clear stable liquid after 9 months' storage at 5°. The following illustration exemplifies the procedure used for the preparation of all the amino derivatives of 2,3,6–TBA.

N–(2,3,6–trichlorobenzoyl)–L–leucine. A 0.5 *M* quantity (65.5 grams) of *L*–leucine was dissolved in 1500 ml. of 1.0 *M* sodium hydroxide solution and chilled to 5°. A 0.5 *M* quantity (122.0 grams) of 2,3,6–trichlorobenzoyl chloride was dissolved in 1500 ml. of benzene and chilled to 5°. The benzene solution was added dropwise during continuous stirring to the alkaline solution of *L*–leucine. The temperature was maintained at 5°; the time required for complete addition of the benzene solution was about 1 hour. The reaction mixture was stirred an additional 3 hours as it warmed up to room temperature. Three ethyl ether extractions were made of the alkaline reaction mixture using 500, 250 and 250 ml. portions of ether. These ether extracts were combined and washed once with 200 ml. of distilled water. This wash water was added to the ether extracted aqueous alkaline reaction mixture and the solution (pH 10.4) was adjusted to a pH of 1.2 with 10% hydrochloric acid; it was allowed to stand for 20 hours at 5°. The crude white crystalline product was filtered off, slurried several times with distilled water, filtered off and vacuum dried at room temperature to constant weight, crude yield 135.0 grams (79.5%).

Purification consisted of extracting in a Soxhlet apparatus for about 20 hours with petroleum ether (b.p. 63–70°) to remove unreacted 2,3,6–TBA; purified yield 122.6 grams (72.3%).

The *D*–leucine, *L*–methionine and *D*–, *L*–, and *DL*–threonine derivatives were purified in the same manner.

Crude *DL*–leucine and *DL*–methionine derivatives were recrystallized from hot ethyl acetate–petroleum ether; the crude *D*–methionine received the same treatment plus one recrystallization from dilute ethanol followed by washings with hot petroleum ether. The crude *DL*–threonine derivative was recrystallized from hot petroleum ether (b.p. 95–127°).

Information on yields, properties and analyses of the 9 amino acid derivatives of 2,3,6–TBA prepared is presented in Table 2. The melting points of some of these compounds were indefinite and in several instances were not improved by additional recrystallizations.

RESULTS AND DISCUSSION

In the preparation of the amino acid derivatives of both dalapon and 2,3,6–TBA most of the compounds in the crude state separated from reaction mixtures as crystalline white solids. Occasionally instead of a white solid an oil was obtained which crystallized on

Table 2. Yields, physical properties and analyses of amino acid derivatives of 2,3,6–trichlorobenzoic acid.

N–(2,3,6–dichlorobenzoyl)–	M.P. (corr.)	Yield, %		Analyses[a]				
				Cl, %		N, %		Optical rotation[b]
		Crude	Refined	Calcd.	Found	Calcd.	Found	$[\alpha]_D^{28}$
L-leucine	129–132	79.5	72.8	31.42	31.58	4.14	4.09	−4.23
D-leucine	148–160	88.5	78.9	31.42	31.70	4.14	4.10	+11.43
DL-leucine[c]	95–102	52.8	37.9	31.42	29.88	4.14	2.95	+0.06
L-methionine	Indefinite	30.8	20.7	29.82	29.86	3.93	3.94	−2.40
D-methionine	60–65	81.3	60.8	29.82	29.82	3.93	3.71	+6.50
DL-methionine	Indefinite	45.7	27.5	29.82	29.49	3.93	3.86	
L-threonine	Indefinite	10.7	4.9	32.57	33.10	4.29	3.13	+21.03
D-threonine	Indefinite	26.0	15.0	32.57	32.46	4.29	4.18	−22.74
DL-threonine	161.164	27.8	27.7	32.57	32.31	4.29	4.28	

[a]Analyses by J. S. Ard.
[b]Concentration 3.00 grams/100 ml. in pyridine; error estimated to be less than ± 0.3°.
[c]Purity considered acceptable because of a 2.99 chlorine/nitrogen ratio compared to a calculated value of 3.00.

prolonged standing at about 5° C. When these did not crystallize they were thoroughly washed with water after decanting the original mother-liquor. When evacuated to constant weight at room temperatures these oils turned to amorphous solids and could then be purified by the usual procedures.

Since the amino acid derivatives of dalapon have subsequently been found to possess moderate water solubility this may account for the low yields obtained in their preparation, especially by Schotten-Baumann techniques. Also, the low yields obtained for the threonine derivatives of 2,3,6–TBA may be for the same reason since they possess considerable water solubility.

No attempts were made to improve yields either by working up mother-liquors or by recovering products from petroleum ether extracts in purification procedures. Reduction in water volumes used in Schotten–Baumann reactions where water solubility is a contributing factor should result in improved yields.

SUMMARY

1. Nine dalapon and nine 2,3,6–TBA amide derivatives of amino acids have been synthesized using the *D*–, *L*– and *DL*– configurative forms of leucine, methionine, and threonine.

2. Most of these compounds were readily prepared, purified and analyzed. Where sharp melting points, and characterizing rotations for *D*– and *L*– optical forms, prevail such derivatives may be useful in the identification of amino acids.

3. Preliminary field tests on *L*–leucine derivatives of dalapon and 2,3,6–TBA have shown the pronounced effect of amino acid coupling upon the selectivity pattern of the parent compounds. These results are in close agreement with past experiences concerned with amino acid compounds of phenoxy herbicides.

4. Biological evaluation of the compounds as selective herbicides

is being made by other investigators and will be published later. Some of the compounds are also being evaluated as nematocides, fungicides, and as anticancer agents.

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Effect of Sucrose on the Toxicity of Several Phenylurea Herbicides to Barley

W. A. Gentner and J. L. Hilton[1]

Several investigators have now reported that phenylurea herbicides inhibit the photochemical activity (Hill reaction) of isolated chloroplasts (1, 5, 6, 7). Many of the phytotoxic symptoms in plants treated with phenylurea herbicides can be explained on the basis that the herbicides have blocked the photosynthetic mechanism. This action has been suggested as the primary cause of toxicity under field conditions (8). The metabolic product of biochemical pathways containing the primary site of action of a herbicide can be applied to barley plants in sufficient quantity to eliminate completely some of the toxic symptoms produced by the herbicide (3, 4). Geoghegan (2) has reported that glucose applications caused an increase in the concentration required for three phenylurea compounds to inhibit growth of *Chlorella vulgaris*. Therefore, a determination of the effect of carbohydrates on the inhibition of growth of barley seedlings in the presence of several phenylurea herbicides seemed of interest.

Materials and Methods[2]

Experiments were conducted in which herbicide-treated barley plants (*Hordeum vulgare*, var. Dayton, CI 9517) were supplied carbohydrate from an exogenous source. The new leaf growth which occurred subsequent to treatment was used as a measure of the protective effect of sucrose against the phytotoxicity of five phenylurea herbicides.

An incomplete factorial experimental design with three replications was used. The experiment was repeated three times.

Five barley seeds were planted ¼ inch deep in perlite contained in 8-ounce plastic containers which had been drilled to accommodate subirrigation. Immediately after planting, the cups were placed in Hoagland's nutrient solution and allowed to imbibe until the perlite was saturated. All containers were placed in a controlled environment room where a day temperature of 80° F and a night temperature of 65° F prevailed. Daylength was maintained at 15½ hours. The intensity of light was ca 1000 foot candles at plant level.

Ten days after seeding, when the plants were 10–12 cm tall and in the two-leaf stage, they were thinned to the three most uniform plants per container. Approximately 5 mm of the leaves of all plants were removed aseptically. One plant in each container received no additional leaf-treatment; the cut leaf tips of the second plant were immersed in 5 ml of distilled water; the cut leaf tips of the third plant

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[2]The authors wish to acknowledge the cooperation of the research personnel of the E. I. duPont deNemours Chemical Company who supplied experimental compounds for these studies.

were immersed in 5 ml of 0.3 M sucrose solution which had been prepared with distilled water and autoclaved. All solutions used in leaf treatments were contained in acid-cleaned 10 ml vials held in place by adjustable wire stands. The leaves were held in position in the vials with a soft cotton plug.

Applications equivalent to $^1/_{32}$, 1 and 32 lb/A of the active ingredient of 3–phenyl–1,1–dimethylurea [fenuron], 3–(*p*–chlorophenyl)–1,1–dimethylurea [monuron], 3–(3,4–dichlorophenyl)–1,1–dimethylurea [diuron], 1–*n*–butyl–3–(3,4–dichlorophenyl)–1–methylurea [neburon], and 3–(3,4–dichlorophenyl)–1–methylurea [DMU] were pipetted on the perlite media at this time.

Plants were watered and the sucrose solutions were changed every second day. Fresh cuts were made on the tips of the first and second leaves of all plants when leaf-treatment solutions were changed.

Plants were harvested 10 days after herbicide application and the fresh weight recorded for leaves appearing subsequent to herbicide treatments. Fresh weight was chosen over dry weight for measurement in order to minimize the possible effect of excessive sucrose accumulation.

RESULTS

Each of the five herbicides inhibited new leaf development when applied at $^1/_{32}$ lb/A. Higher rates of herbicide application resulted in greater inhibition of new leaf development or the death of the barley plants treated with fenuron, monuron, diuron, and DMU. Lethal concentrations of the herbicides manifested themselves by a wilting of the entire barley plant in 4–6 days followed by chlorosis and desiccation after about 10 days. Phytotoxic symptoms resulting from herbicide application to plants which received no leaf-treatment and plants which had leaves immersed in water were similar; but herbicide-treated plants which had leaves immersed in water developed the above symptoms several days later than plants which received no leaf-treatment. With the exception of neburon, the complete inhibition of new leaf growth and subsequent death of plants receiving no leaf-treatment and water leaf-treatment was achieved with the 1 lb/A rate of all compounds studied. Neburon applications frequently completely inhibited new leaf growth, but the characteristic wilting and desiccation symptoms of feuron, monuron, diuron and DMU did not occur even at rates as high as 32 lb/A. Under the described light conditions check plants which received sucrose leaf-treatments had increased growth when compared to check plants which received water or no leaf-treatment (Av. weight per plant of new growth as determined by fresh weight 208 mg, 86 mg, and 101 mg, respectively).

The effect of the sucrose solution and of water applied to the first two leaves of herbicide-treated barley plants are compared in Figure 1. The fresh weight of the above-ground plant parts which developed subsequent to herbicide application is presented as the percentage of the new growth obtained with plants receiving the same leaf-treatment but no herbicide. Sucrose reduced the amount of herbicide-inhibition of new growth obtained in the presence of the individual

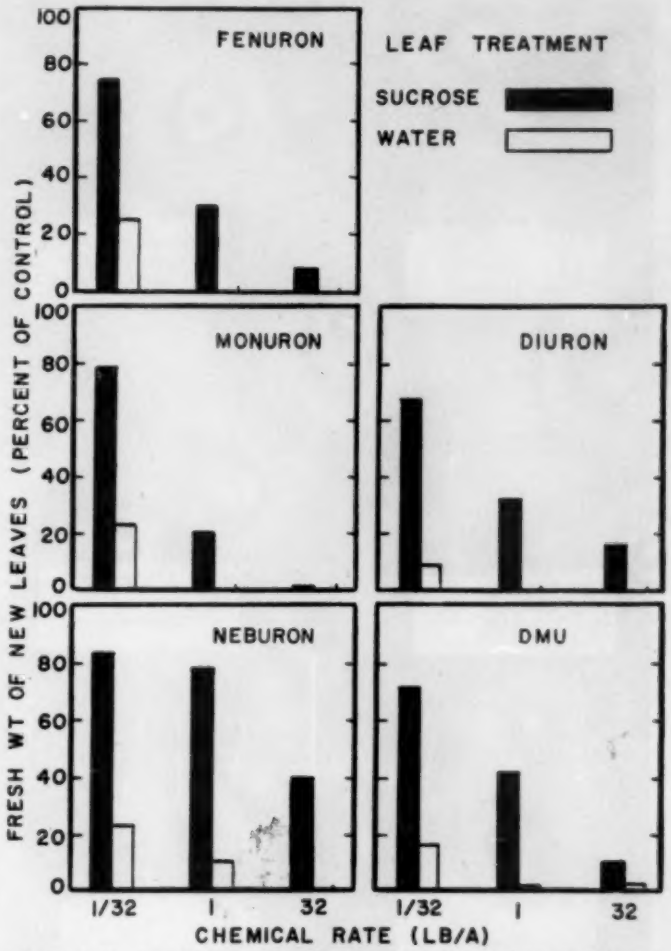

Figure 1. Fresh weight of new leaves which appeared on barley seedlings 10 days after herbicide application. Tips of old leaves (first two leaves) were removed and placed in water or sucrose solution. Values are expressed as percentage of new leaf growth of plants receiving same leaf-treatment but no herbicide.

herbicides. Figure 2 shows the appearance of barley seedlings treated with 1 lb/A of fenuron, monuron, diuron, neburon, and DMU when plants received through the leaf (from left to right); (a) no treatment, (b) water, and (c) sucrose solution. The responses illustrated in Figures 1 and 2 were evident 10 days after treatment.

The inhibition of new leaf development resulting from the applications of the herbicides included in these studies was greatly reduced when the first two leaves of the herbicide-treated plants were immersed in sucrose solutions; however, the sucrose treatments became

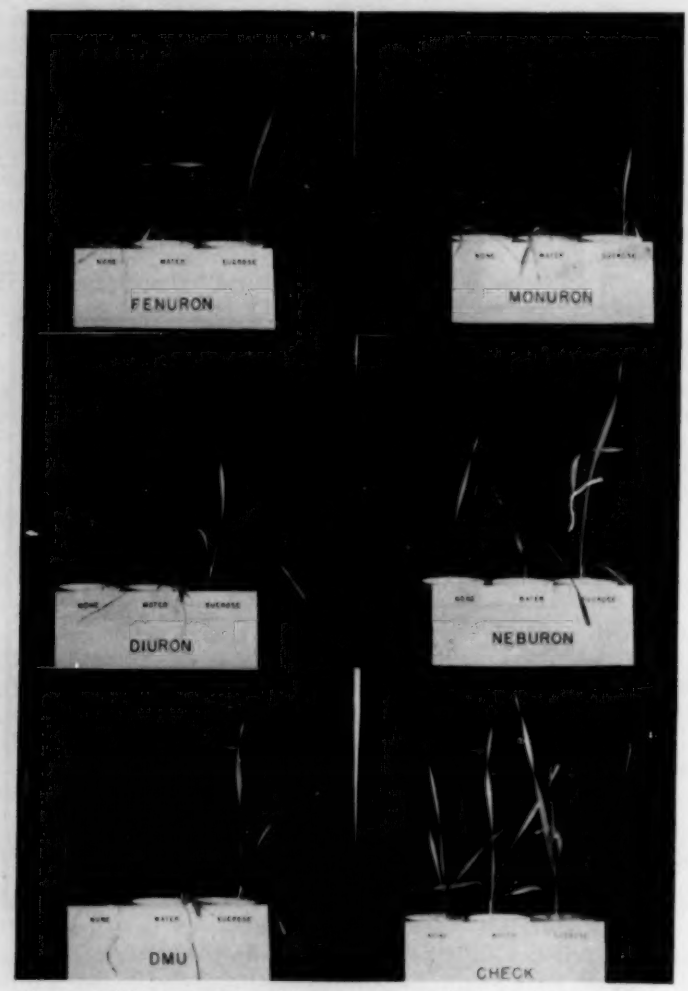

Figure 2. The responses of barley plants, 10 days after treatment, receiving (from left to right) no leaf treatment, leaf tips immersed in water, and leaf tips immersed in sucrose solution to the 1 lb/A rate of several phenylurea herbicides.

less effective as the herbicide concentrations increased. These results provide additional evidence that the toxic symptoms produced by five phenylurea herbicides in barley are primarily a consequence of a herbicide-induced deficiency of photosynthate. The decreased effectiveness of sucrose against higher rates of herbicide application suggests that metabolic reactions other than photosynthesis may be sensitive to these herbicides.

SUMMARY

Sucrose furnished to barley plants through cut leaf tips kept plants alive and growing in the presence of lethal concentrations of fenuron, monuron, diuron, neburon, and DMU.

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Field Bindweed *(Convolvulus arvensis)* Control With Soil Applications of Phenoxy Herbicides[1]

A. F. Wiese and H. E. Rea[2]

The possibility of using 2,4–D as a soil sterilant was investigated by Crafts (2) in 1949. He concluded that 2,4–D may be useful under very dry conditions. In 1954, Shafer (7) reported several years work using soil applications of 2,4–D for control of perennial weeds. Since that time many research workers have evaluated 2,4–D and other phenoxy herbicides for the control of bindweed and other perennial weeds. Results of these trials varied from excellent to poor.

The period of toxicity to crops after heavy soil applications of 2,4–D for perennial weed control has usually been less than 1 year (7, 9). The persistence of 2,4–D in soils was reduced by high moisture, high temperature and high aerobic bacterial count (1, 2, 4, 5). The toxicity of 2,4–D in soils was not appreciably affected by formulation (3). Stone and Rake (8) noted that soil organisms utilized 2,4–D as a nutrient. They were able to increase the residual period of 2,4–D in soils by reducing soil micro-organism activity with sodium borate.

Phenoxy compounds appeared to have promise for perennial weed eradication. Studies were conducted to evaluate several chemicals for this purpose and to determine factors which enhanced or detracted from their effectiveness.

Methods and Materials

Field studies.

A number of herbicides were applied in water sprays to square rod plots at 80 lb/A in triplicated randomized blocks in July and November, 1954; May, July and November, 1955; June, August and November, 1956. The herbicides applied were ethanol and isopropyl series amines of 2,4–dichlorophenoxyacetic acid (2,4–D); propylene glycol butyl ether ester of 2,4–D; emulsifiable acid formulation of 2,4–D; sodium 2,4–dichlorophenoxyethyl sulfate (sesone); triethanol amine of 2,4,5–trichlorophenoxyacetic acid (2,4,5–T); propylene glycol butyl ether ester of 2,4,5–T; 2,4,5–trichlorophenoxyethyl sulfate (2,4,5–TES); an amine salt of 2–(2,4,5–trichlorophenoxy) propionic acid (silvex); propylene glycol butyl ether ester of silvex; and 3–amino–1,2,4–triazole (amitrole). Anhydrous borax, containing 61.5% boron trioxide (Concentrated Borascu) was applied at 2560 lb/A and a DBM containing 7.5% 2,4–D and disodium tetraborate pentahydrate and decahydrate to total 41% boron trioxide (DB Granular) was applied at 640 lb/A. The treatments were evaluated

[1]Work was conducted cooperatively between Texas Agricultural Experiment Station and the Western Soil and Water Management Research Branch, SWCRD, ARS, USDA. Approved as TAES T.A. 3339.

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for bindweed control by counting the number of bindweed plants remaining in the central square meter area of each plot approximately one year after the chemical application.

The period of residual toxicity from the 1954 and 1956 applications was determined by planting sorghum in soil samples taken at three month intervals from the surface of each plot. A chemical was considered toxic if sorghum growth was stunted or prevented on at least two of the three replications in each experiment.

The soil type was Pullman silty clay loam, and the average yearly rainfall for the area is 18 inches.

Greenhouse study.

The effect of soil moisture on the decomposition of phenoxy herbicides used in the field studies was determined by spraying 80 lb/A of the chemicals to soil in greenhouse pots. Part of the pots were maintained wet and the others were kept dry until planted to sorghum. Sorghum was planted at 15, 30, 45, 60 or 90 days after chemical application. If the sorghum grew normally the chemicals were assumed to have decomposed. The dates of planting were main plots, and the herbicides were the sub-plots in a triplicated split-plot design.

RESULTS AND DISCUSSION

Field studies.

Bindweed counts made approximately one year after chemical application showed that the amine and ester formulations of silvex gave the best bindweed control, 89 and 87 percent, respectively (Table 1). Formulations of 2,4,5–T and 2,4,5–TES were next in

Table 1. Bindweed plants remaining approximately one year after soil applications of herbicides.

Herbicide	Lb/A	Plants per square meter, one year after application on								Av.	Av. pct control
		7–29 1954	11–2 1954	5–4 1955	7–27 1955	11–15 1955	6–6 1956	8–16 1956	11–14 1956		
Silvex ester	80	3.3	4.0	7.6	6.0	10.3	0.0	3.6	2.6	4.6	87
Silvex amine	80	3.0	4.3	0.0	1.3	21.0	0.6	0.3	0.6	3.9	89
2,4,5–T ester	80	5.6	9.6	7.0	4.0	7.0	9.6	13.0	20.6	9.5	73
2,4,5–T amine	80	9.0	9.3	10.3	1.0	3.3	13.3	7.3	17.0	8.8	75
2,4,5–TES	80	15.6	13.6	7.0	1.0	0.3	1.3	9.0	17.3	8.1	77
2,4–D ester	80	7.0	19.0	0.0	29.3	9.3	23.3	12.0	6.6	13.3	63
2,4–D amine	80	6.0	16.3	5.0	22.0	13.0	23.0	15.0	7.0	12.1	66
2,4–D acid	80	16.0	15.3	13.3	38.6	21.3	29.6	16.3	16.4	20.8	42
Sesone	80	16.0	18.3	7.6	31.0	13.0	18.3	19.6	5.6	16.2	54
Amitrole	80	16.3	25.3	51.0	34.6	32.9	45.3	20.0	16.3	30.2	15
DBM	640	10.0	13.3	2.0	23.3	8.3	8.6	15.0	9.6	11.2	69
Anhydrous borax	2560	2.6	0.3	12.3	8.6	1.3	19.0	0.0	3.3	5.9	83
Untreated		28.6	31.6	51.0	38.6	39.3	36.6	33.3	25.3	35.6	0

LSD 0.05 for chemicals within dates = 14.4
LSD 0.05 for average of chemicals = 5.1

effectiveness, followed by 2,4–D formulations and sesone. Amitrole did not appreciably reduce the bindweed stand. Anhydrous borax averaged 83 percent control one year after application. However,

this herbicide usually does not reach its maximum effectiveness until two years have elapsed. The DBM at 640 lb/A gave 69 percent control.

The period of chemical toxicity in the surface soil of the field plots was determined by taking samples from each plot and planting them to sorghum in the greenhouse. A preliminary study showed that soil concentrations of the various chemicals in excess of 50 ppm prevented sorghum germination and growth. Concentrations as low as 10 ppm stunted sorghum growth. Anhydrous borax caused the longest period of soil toxicity to sorghum and was still preventing sorghum growth when sampling was concluded (Table 2). Silvex

Table 2. Months of chemical toxicity to sorghum in the field studies.

Herbicide	Lb/A	Months of sterility following application on				
		7–29–54	11–2–54	8–16–56	11–14–56	Average
Silvex ester	80	24	30	15	18	21.8
Silvex amine	80	21	21	15	18	18.8
2,4,5–T ester	80	9	9	3	6	6.8
2,4,5–T amine	80	15	12	3	12	10.5
2,4,5–TES	80	15	18	3	6	10.5
2,4–D ester	80	3–	6	3	3	3.8–
2,4–D amine	80	3	6	3	3	3.8
2,4–D acid	80	3–	6	3	3	3.8–
Sesone	80	3–	6	3	6	4.5
Amitrole	80	3–	6	3	3	3.8–
DBM	640	9	12	6	12	9.8
Av. (excl. anhyd. borax)		8.7	12.0	5.5	8.2	
Anhydrous borax	2560	33+	30+	21+	18+	25.5+
		Rainfall after application				
3 months		2.42	0.21	1.99	0.46	
6 months		2.60	0.73	2.45	6.64	
12 months		8.95	12.61	19.19	21.52	

ester caused an average of 21.8 months toxicity. Formulations of 2,4,5–T and 2,4,5–TES caused from 6 to 10 months toxicity, and 2,4–D formulations persisted about three months. DBM persisted for 9.8 months. The longest average period of residual toxicity from all chemicals, 12 months, occurred from the November, 1954 applications. The rainfall for the 12 month period following application was only 12.61 inches. Only 5.5 months of toxicity occurred from the August, 1956 application when rainfall during the year after application was 19.19 inches. Because little rainfall occurs during the winter, fall applications persisted longer than summer applications. The correlation coefficient between the number of plants remaining, (Table 1) and the months of soil toxicity, (Table 2) for the 1954 applications and the last two applications in 1956 was —0.623. This indicates that a considerable portion of herbicide effectiveness was related to the period of residual toxicity.

Greenhouse study.

Ester and amine formulations of silvex, 2,4,5–T and 2,4–D as well as amitrol and 2,4,5–TES did not disappear from the soil surface in

a 90 day test period when the soil was maintained dry although temperatures in the greenhouse frequently reached 120 degrees. Where the soil was wet, silvex formulations persisted in excess of 90 days and 2,4,5–T formulations persisted for 90 days. Amitrole and formulations of 2,4–D persisted 15 days or less.

These studies indicate that soil applications of silvex were more effective against bindweed than other phenoxy herbicides. The effectiveness of silvex was associated with a relatively long period of residual toxicity in the soil. The persistence of phenoxy herbicides in the soil was controlled by rainfall. The chemicals remained in the soil for long periods under dry conditions and disappeared rapidly when the soil was wet. Because it is difficult to anticipate future rainfall conditions, single applications of phenoxy herbicides for bindweed control probably will give erratic results. However, eradication of bindweed has been achieved by applying silvex to soil and to bindweed crowns several times during a season (6).

SUMMARY

The effectiveness of soil applications of several phenoxy herbicides, amitrole, and two boron formulations for eradicating field bindweed was evaluated. Formulations of silvex gave the most satisfactory control. The effectiveness of silvex appeared to be related to its ability to persist in the soil longer than other phenoxy herbicides. The period of residual toxicity of phenoxy herbicides was increased under dry soil conditions.

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The Control of Weeds Around Young Apple Trees[1]

J. S. Leefe and R. P. Longley

In order to protect young apple trees, in the years immediately following planting, from competition for moisture and plant food it is a generally accepted practice to keep the area around the trees free of weeds. The traditional method of doing this has been to clean cultivate, the area next to the trees being kept clean by hand labour.

In the apple growing area of Nova Scotia couchgrass *(Agropyron repens)* is frequently the principal weed competing with new planted apple trees although annual, biennial and other perennial weeds are often a problem.

The control of grass around apple trees with dalapon has been reported by Hall (1), Harrison (2) and Leefe (3).

The object of the experiment described here was a comparison of chemical and cultural methods of controlling vegetation around young apple trees.

Materials and Methods

Two-year-old Secor trees on Malling I rootstocks were planted in land heavily infested with couchgrass. The trees were planted in rows 10 feet apart with 5 feet between the trees in the rows. Both chemical and cultural treatments were confined to a strip four feet wide with the trees in the center. The remaining area, between the rows was kept under clean cultivation. Each plot consisted of four trees.

The eight treatments (Table 1) were replicated four times in randomized blocks. The herbicides were applied as sprays, using a hand boom, at 30 psi with fan type nozzles. The mechanical hoeing operation was performed with a Friday Automatic Rotary Hoe[2] (Figure 1). The hoeing device on this machine consists essentially of a

Table 1. Estimate of undertree vegetation July 23, 1959.

Treatment	Ground cover, per cent	Plant species
1. Dalapon[a] 7.4 lb/A. .	100	*Daucus carota, Vicia cracca, Achillea millefolium, Solidago* sp.
2. Dalapon 14.8 lb/A. .	100	*Daucus carota, Achillea millefolium, Chrysanthemum leucanthemum, Raphanus raphanistrum, Chenopodium album.*
3. Dalapon 7.4 lb/A plus 2,4–D[b] 1 lb/A	100	*Solidago* sp., *Stellaria graminea, Rumex acetosa, Chrysanthemum leucanthemum.*
4. Dalapon 14.8 lb/A plus 2,4–D 1 lb/A	85	*Stellaria graminea, Solidago* sp., *Rumex acetosa, Raphanus raphanistrum, Chenopodium album Polygonum scabrum.*
5. Amitrole[c] 5 lb/A. . .	50	*Agrostis alba, Raphanus raphanistrum, Chenopodium album.*
6. Amitrole 10 lb/A. .	20	*Agrostis alba, Chenopodium album.*
7. Mechanical hoeing. .	—	No observations were made. All vegetation was removed mechanically 4 times per season.
8. Hand hoeing.	—	

[a]Dowpon, 74% dalapon.
[b]Dimethylamine salt.
[c]Aminotriazole Weed Killer, 50% amitrole.

[1]Contribution No. 1024 from the Research Station, Canada Agriculture, Kentville, Nova Scotia.

[2]Manufactured by Friday Tractor Co., Hartford, Michigan.

Figure 1. Mechanical hoe in operation.

revolving disc, with teeth on the lower side, mounted at the end of an arm, controlled hydraulically by the operator of the machine. The machine is provided with a safety device which stops the disc should it come too close to a tree. Hand cultivation was done with a standard field hoe.

Herbicides for vegetation control were applied once per season when growth of undertree weeds was well started, about the time apple trees were in full bloom. They were applied in 50 gal/A of water, sufficient to wet all undertree vegetation.

Machine and hand hoeing operations were performed as often as necessary to keep the area in the treated strip clear of vegetation until tree growth had stopped for the year. This required four operations per season.

Trunk cross-section was taken as the index of tree size. It was measured one foot above ground level, at the end of each growing season, 1956–1959 inclusive. Estimates of undertree vegetation were made at the end of July in each year and the trees were carefully examined for symptoms of herbicide injury.

RESULTS

Vegetation control.

Although at the beginning of the experiment the land was heavily infested with couchgrass (75–90% ground cover) all the chemical

treatments reduced it to a negligible amount during the first season. Subsequent annual treatments practically eliminated couchgrass from the chemically treated areas.

The mechanical and hand hoeing treatments kept the couchgrass under control except for a collar of grass around the trunk of each tree where the mechanical hoe was used. The couchgrass was not killed as it was in the herbicide treated plots.

On the dalapon treated plots a dense growth of broadleaved weeds developed during the first season and this infestation became progressively worse with each year. The addition of 2,4–D to the dalapon only slightly reduced the infestation of broadleaved weeds.

When amitrole was used at the lower rate fair control of all types of vegetation was obtained and at the higher rate the control was almost complete.

The final estimates of undertree vegetation, made in late July of the last year of the experiment, are given in Table 1. The appearance of annuals like lambsquarters *(Chenopodium album)* and wild radish *(Raphanus raphanistrum)* in some of the herbicide treated areas is due to the fact that these weeds germinated after the sprays were applied and that there was space for them to develop. In plots getting the lower rate of dalapon the concentration of biennial and perennial weeds was so great that there was no space for late germinating annuals. The addition of 2,4–D to the dalapon is associated with the appearance of chickweed *(Stellaria graminea)* and sorrel *(Rumex acetosa)* both of which are resistant to 2,4–D.

Tree growth.

The tree sizes, as measured by the cross-section of the trunk, are given in Table 2. The treatments are arranged in the table in order

Table 2. Tree size expressed as trunk cross-section. Mean of 4 replicates.

Treatment	Area of trunk cross-section, cm^2			
	1956	1957	1958	1959
1. Dalapon 7.4 lb/A	1.63	2.68	4.84	7.75
3. Dalapon 7.4 lb/A +2,4–D	1.84	3.19	6.06	9.94
2. Dalapon 14.8 lb/A	1.75	3.46	6.41	10.50
4. Dalapon 14.8 lb/A +2,4–D	1.89	3.73	7.19	11.80
7. Mechanical hoe	1.84	4.30	9.25	14.69
5. Amitrole 5 lb/A	1.83	4.71	9.44	15.44
6. Amitrole 10 lb/A	2.09	5.19	10.28	16.38
8. Hand hoe	2.17	5.66	11.82	18.53
Standard error of a treatment mean	0.19	0.56	1.15	1.53
Significance of differences based on Duncan's range test at the 5% level.	None significant 1956			[8 and 1,3,2,4 / 6 and 1,3,2 / 5 and 1 / 7 and 1] 1957–58–59

of the size in the final year of measurement and it will be noted that this size order is the same in all years but the first, when there were no significant differences.

The tree size appears in the same order as the density of undertree vegetation given in Table 1 insofar as the herbicide treated plots

are concerned. The mean tree size in the mechanically hoed treatment is less than that in plots getting the lower rate of amitrole.

Based on Duncan's range test (4) hand hoeing produced significantly larger trees than were produced in plots treated with dalapon or dalapon plus 2,4–D but not significantly larger than those in plots treated with amitrole or machine hoed. The trees in the plots treated with the higher rate of amitrole were significantly larger than those in the plots treated with dalapon alone or the lower rate of dalapon plus 2,4–D, and the trees in the plots treated with the low rate of amitrole or machine hoed were significantly larger than the ones in the plots treated with the low rate of dalapon alone. It may be noted that the variance ratios in the analysis of variance of tree size were significant at the .01 level in the last three years of the test.

With the exception of an occasional leaf showing the characteristic whitening associated with amitrole no herbicide injury symptoms were observed on the trees.

DISCUSSION

The experiment was originally designed to compare chemical with cultural methods of controlling couchgrass in land planted to young apple trees. It became apparent after the first season that a herbicide that controlled couchgrass only would be far from satisfactory. Destruction of the couchgrass by dalapon encouraged a dense growth of broadleaved weeds. The addition of 2,4–D to dalapon resulted in the appearance of weeds resistant to 2,4–D.

Amitrole because of its wide spectrum of herbicidal activity, proved to be the best herbicide for overall vegetation control and at the higher rate almost completely eliminated weedy growth.

Tree growth appears to be directly related to vegetation control and there is no evidence that it was adversely affected by the herbicides. In fact it increased with the amount of herbicide used (Table 2). Trees in the mechanically hoed areas did not make as good growth as those in the amitrole treated areas and the difference, although not statistically significant, was consistent in all years. It seems likely that the use of the mechanical hoe may have resulted in some destruction of tree roots near the surface. The trees in areas kept free of vegetation by hand hoeing were always larger and grew more rapidly than those in the other treatments. The differences between them and the trees in the mechanically hoed or amitrole treated areas, however, were not significant. The relative cost advantage of one method over the other would vary according to cost of labour and materials. However, as measured by weed control and tree growth, one treatment of amitrole per season here compares very well with four hand hoeing treatments. To treat a 6-foot diameter circle around 50 trees at the high rate of amitrole would require about 10 ounces of material, and at prevailing prices this would amount to 2½–3 cents per tree.

SUMMARY

In a four season comparison of cultural and chemical methods of controlling vegetation under young apple trees the greatest tree

growth and largest trees were produced by hand hoeing. The trees were not, however, significantly larger in growth rate or final size than those in areas where vegetation was controlled by amitrole sprays or by mechanical hoeing. When dalapon sprays were used couchgrass control was adequate but the areas under the trees became infested with broadleaved weeds. The addition of 2,4–D to the dalapon did not materially improve vegetation control and the trees in the dalapon and dalapon plus 2,4–D treated areas were significantly smaller than those in the hand hoed areas.

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Mesquite Root Inhibition Tests to Study Inhibitory Activity, Absorption and Translocation of 2,4–D and 2,4,5–T[1]

RICHARD BEHRENS[2] and HOWARD L. MORTON[3]

THE technique used in the tests herein reported is based on the inhibition of root elongation by growth substances, an exceedingly sensitive response. Two procedures are used in the application of the growth substances. The first consists of applying known amounts of test compounds to the root bathing solution of mesquite seedlings, and the second procedure consists of applying these compounds to the cotyledons. After addition of the growth regulator to the solution subsequent root inhibition is dependent on absorption by the root tissue and accumulation in an active form at the site of action. In cotyledonary applications, translocation as well as absorption is necessary before inhibition of root elongation will occur. It was the objective of this study to investigate various possibilities for using these procedures in herbicide absorption and translocation experiments.

The use of root inhibition as a quantitative test for growth hormones was suggested by Lane (13) in 1936. The inhibition of *Avena* root elongation was employed by Bonner and Koepfli (5) in obtaining dosage response curves suitable for making quantitative estimates of indoleacetic acid (IAA). Thereafter, extensive use was made of this response in studying the action and activity of growth substances. Reviews by Aberg (1), Burstrom (6), and Torrey (18) summarize most of the root inhibition experiments, criticize the technique and discuss the significant findings.

In most root inhibition experiments, the compounds have been added to the root bathing medium. However, IAA applications to stumps of shoots (17) and the endosperm (5) of *Avena* were found to retard root growth. On the other hand, similar applications to *Pisum* and *Zea mays* (17) failed to inhibit root development. Crafts (7) reported that measurements of root injury could not be obtained when small dosages of 2,4–D were applied to cotton leaves though large doses killed the roots.

Van Overbeek (19) concludes that plant growth regulators are absorbed and translocated in the same manner as other organic compounds. Both direct and indirect evidence indicating the translocation of the intact molecule of phenoxy compounds has been presented by Crafts (7), Fang, et al. (9), Hay and Thimann (12), and many others. On the basis of their findings, it was assumed in the

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present experiments that mesquite root growth inhibition was a direct response to the accumulation of 2,4–D and 2,4,5–T in the elongating region of the root.

MATERIALS AND METHODS

The procedure used in the root inhibition tests was patterned after that of Barton and Trelease (3). Scarified seeds of mesquite *(Prosopis juliflora* var. *glandulosa)* were germinated in Petri dishes on filter paper at 30° C for 36 to 48 hours; then transferred to 250 ml. wide-mouth jars filled with the bathing medium. Preliminary tests indicated that root growth was equally vigorous in these short-term experiments whether tap water or nutrient solution was used as a bathing medium. Also, aeration of the root bathing solution proved to be unnecessary since the rate of root elongation was the same with and without aeration. Fifteen seedlings per jar were suspended by the cotyledons through perforations in paraffined, hardware-cloth jar covers (Figure 1). After placement the seedlings were

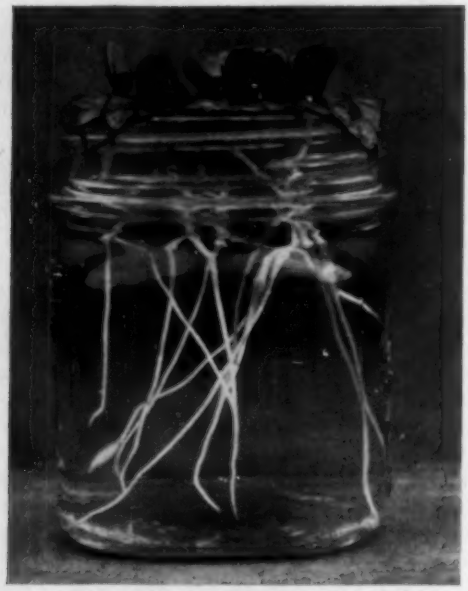

Figure 1. Suspension of mesquite seedlings in root bathing medium by means of paraffined hardware cloth.

illuminated for 16 to 24 hours at room temperature with 150-watt, reflector flood lamps positioned 18 to 24 inches from the plants; then seedlings were thinned to 12 per jar and the length from the tip to the prominent root collar was recorded.

In solution root-inhibition tests, the bathing medium was replaced by a tap water solution of the test compound immediately after completion of the root length measurement. The roots were remeasured approximately 48 hours after the first measurement and the effect on elongation during this period was used to estimate the inhibitory activity of a compound.

In the cotyledonary applications a measured drop of the test compound was applied to the lower surface near the tip of a single cotyledon. A micrometer-driven syringe (15) was used to apply 5.25- or 2.06-microliter droplets. The 5.25-microliter droplets were too large to remain in place on the cotyledon and flowed to the base of the cotyledon and accumulated there. The 2.06-microliter droplets either flowed or remained at the point of application, depending on the surface tension of the carrier.

The second root measurement was made either 24 or 48 hours after treatment when cotyledonary applications were made. However, more frequent and repeated measurements were necessary in some of the tests.

For statistical purposes an individual jar of 12 seedlings was considered an experimental unit and 3 to 6 replicates were used in a completely randomized design. Data from the root length measurements are in terms of growth as percent of check unless otherwise stated.

The 2,4,5–trichlorophenoxyacetic acid (2,4,5–T) was prepared by re-crystallization of technical grade material from 30 percent ethanol. The 2,4–dichlorophenoxyacetic acid (2,4–D) was purchased as a purified compound and was used without further purification.

RESULTS AND DISCUSSION

1. *Addition of 2,4–D and 2,4,5–T to the root bathing medium.* The relative inhibitory activity of 2,4–D and 2,4,5–T was determined using 0.10-, 0.25-, 0.50, 1.00-, 2.00-, and 4.00-ppm concentrations in the root bathing medium. A concentration of 2,4,5–T three to four times greater than 2,4–D was required to induce a given level of root inhibition (Figure 2). The comparison was repeated several times using concentrations ranging from 0.01- to 4.00-ppm and in all cases 2,4–D was the more inhibitory compound.

Leaper and Bishop (14) found little or no difference in the degree of root inhibition induced by 2,4–D and 2,4,5–T using 1- to 1000-ppm concentrations and *Lupinus albus* as the test plant. Blair (4) reported that 2,4,5–T was more inhibitory to the root growth of germinating mesquite *(P. juliflora* var. *velutina)* than 2,4–D at concentrations above 2-ppm. On the other hand, 2,4–D caused the greater root suppression at 1-ppm in his tests. Any one of several factors might be responsible for the variance in the relative inhibitory activity of 2,4–D and 2,4,5–T found in the tests described herein and those reported by Blair (4) and Leaper and Bishop (14). Although the inhibition of root elongation was used as a measure of activity in all cases, the tests differed as to plant species or variety, root bathing medium, herbicide concentration and technique. The differences in

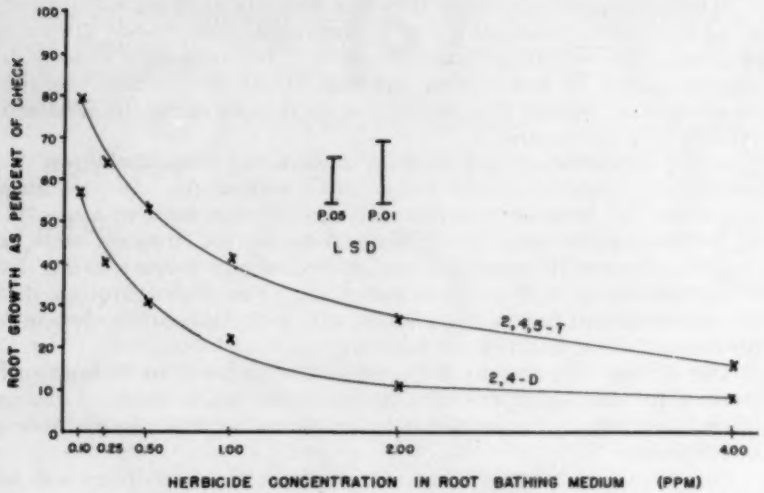

Figure 2. Inhibition of mesquite root elongation when 2,4–D and 2,4,5–T were added to the root bathing medium.

response of plant species to herbicides are well known. It seems likely that the high concentrations used by Blair (4) and Leaper and Bishop (14) might have masked differences that would have been evident at lower concentrations.

Several workers (2, 8, 16, 20) have suggested that the uptake of weak acids by plant tissues is, for the most part, as undissociated molecules. Since the dissociation constants (pK) calculated for 2,4,5–T and 2,4–D are 3.46 and 3.22,[4] respectively, the inhibitory effectiveness of 2,4–D over that of 2,4,5–T would be even greater on the basis of the undissociated molecule concentration in the mesquite root inhibition test. This rules out the possibility that 2,4–D solutions contain greater concentrations of undissociated molecules which would facilitate uptake.

2. *Cotyledonary applications of 2,4–D and 2,4,5–T.* In an effort to determine the relative effectiveness of absorption and basipetal translocation of 2,4–D and 2,4,5–T, applications ranging from 0.0525 to 2.62 micrograms were applied to the cotyledons of mesquite seedlings in 5.25-microliter drops with 95 percent ethanol as carrier. Approximately 10 times as much 2,4–D was required to induce a given level of root growth inhibition (Figure 3). The dosage response curves of Figure 3 demonstrate also, that it is possible to make quantitative comparisons of cotyledonary applications using this technique. Therefore, it should be useful as a means of investigating factors affecting absorption and basipetal translocation of various growth substances.

In the cotyledonary treatments the relative activity of 2,4–D and 2,4,5–T was the reverse of that found when these compounds were

[4]Unpublished data to be included in a subsequent paper.

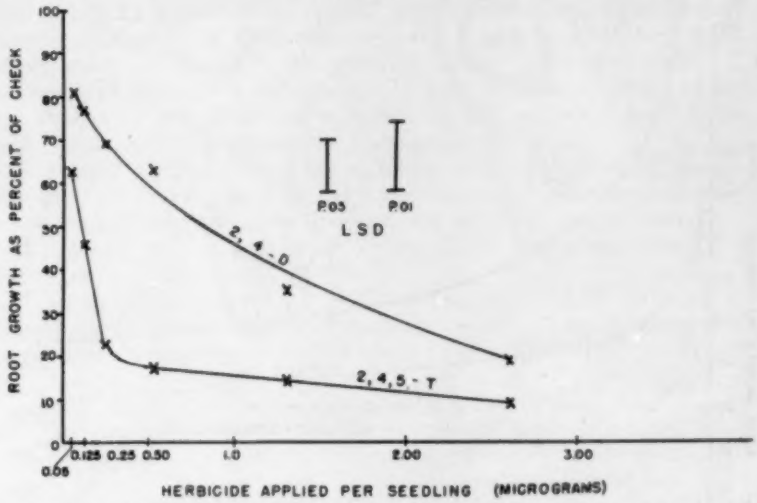

Figure 3. Inhibition of mesquite root elongation when 2,4–D and 2,4,5–T were applied to the cotyledons.

added to the root bathing medium. Since cuticular penetration and basipetal translocation were factors in this test and not in the previous one, it seems probable that the greater activity of 2,4,5–T was due to selectivity factors in absorption, translocation, or both.

The influence of three carriers, 75 percent ethanol, diesel fuel and 60 percent acetone–40 percent nontoxic oil,[5] on the cotyledonary absorption of 2,4,5–T was determined by means of root growth tests. Droplets of 2.06 microliters were used to apply 0.026, 0.103, 0.412 and 1.65 micrograms of 2,4,5–T to mesquite seedlings.

The acetone-nontoxic oil carrier was by far the most effective carrier. When diesel fuel and ethanol were used, 5 and 25 times as much 2,4,5–T was required to induce a corresponding degree of inhibition (Figure 4). Observations indicated that the carriers were not readily absorbed by the cotyledons. Appreciable penetration by diesel fuel would have resulted in rapid contact kill of the plant tissue. However, contact injury was evident on less than 1 percent of the cotyledons treated with this carrier. In instances where contact injury was evident, entry of the diesel fuel was associated with epidermic ruptures incurred in handling the seedlings. Stomatal entry of these carriers was not apparent though stomata are numerous on both upper and lower surfaces of the cotyledons. The magnitude of 2,4,5–T absorption appeared to be associated with the surface area covered by the drops. Ethanol droplets remained at the point of application, on the under surface near the tip of the cotyledon, and tended to cover a relatively small area. The diesel fuel drops flowed from the apex to the base of the cotyledons; in most instances wetting

[5]S/V Sovaspray 100—a petroleum oil with low phytotoxicity supplied through the courtesy of the Socony-Vacuum Oil Company, Incorporated.

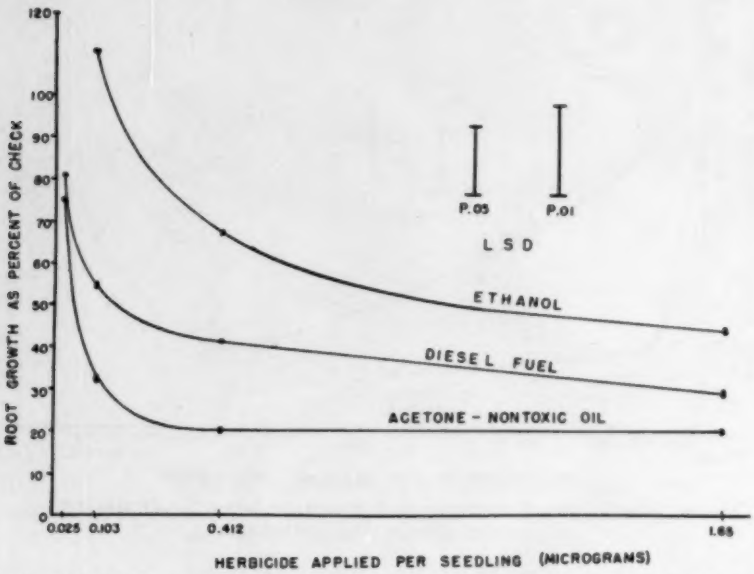

Figure 4. Inhibition of mesquite root elongation by cotyledonary application of 2,4,5–T in ethanol, diesel fuel and acetone-nontoxic oil carriers.

several times more surface than the ethanol carrier. Acetone-nontoxic oil drops covered by far the greatest area. After flowing to the cotyledon base, this mixture ascended the opposite (upper) surface and spread over most of that area, also.

The length of time between cotyledonary applications of 2,4–D and 2,4,5–T and the development of root growth inhibition was determined by making repeated root length measurements at various intervals after treatment. Preliminary tests indicated that root growth inhibition did not develop during the first 4 hours after application of the compounds to the cotyledons. In a subsequent test, root length was measured at 0, 4, and 6 hours after the application of 4 micrograms of 2,4–D and 2,4,5–T per plant. Again there was no evidence of root growth inhibition during the first 4 hours (Table 1). Measurements made six hours after treatment showed a reduction in root growth to 62 and 54 percent of check for 2,4–D

Table 1. Root growth of mesquite seedlings following 4-microgram cotyledonary applications of 2,4–D and 2,4,5–T.

Growth period (hours from application)	Root growth as per cent of check during each period	
	2,4–D	2,4,5–T
0–4	105	94
4–6	62**	54**

**Significantly different from check, P=0.01.

and 2,4,5–T, respectively, during the 4 to 6 hour period. Radioisotope studies (7, 9) have demonstrated that 2,4–D can be found in small amounts throughout plants 2 hours after treatment. The necessity of accumulating inhibitory concentrations in the elongating region of the root may account for the greater length of time from treatment to response in this experiment.

Repeated applications were made to the cotyledons of mesquite seedlings to determine whether impairment of translocation might occur. Single and repeated applications were compared using 2,4–D and 2,4,5–T as the inhibitory compounds. Repeated applications were spaced at 24-hour intervals.

A single application of 0.5-micrograms of 2,4,5–T reduced root growth to 45 percent of check during the first 24-hour interval and 30 percent during the second 24 hours (Table 2). When the mesquite

Table 2. Root growth as percent of check following single and repeated applications of 2,4,5–T to mesquite cotyledons.

Hours after initial application	Root growth as per cent of check	
	Single 0.5 ugm application	Initial plus 24-hour 0.5–ugm applications
0–24	45	—
24–48	30	17
48–72	31	13
72–120	57	23

seedlings received two 0.5-microgram treatments, root growth during the second 24 hours was reduced considerably more, to 17 percent of check. The greater effectiveness of the two applications persisted until the test was terminated, 120 hours after the initial application.

In a similar but more extensive test, 0.5- and 1.0-microgram applications of 2,4–D were used. Two 1.0-microgram applications reduced root growth during the second 24 hours to 42 percent of check versus 72 percent in the case of a single application (Table 3). Two 0.5-

Table 3. Root growth as percent of check following single and repeated application of 2,4–D to mesquite cotyledons.

Hours after initial application	Root growth as per cent of check			
	Single 1.0–ugm application	Initial and 24 hr. 1.0–ugm applications	Initial and 24 hr. 0.5–ugm applications	Initial, 24 and 48 hr. 0.5–ugm applications
0–24	51	—	—	—
24–48	72	42	72	—
48–72	113	52	88	62

microgram treatments induced the same level of inhibition as an initial application of 1.0-microgram. A third 0.5-microgram application made 48 hours after the initial application resulted in a further reduction in root growth.

From these results it seems probable that basipetal translocation

was not greatly disrupted by the introduction of 2,4–D or 2,4,5–T into the mesquite seedlings. The root growth rate approximated that of the check 48 to 72 hours after treatment with 1.0-microgram of 2,4–D indicating normal basipetal translocation from the cotyledons of metabolites essential for root growth. The effective downward movement of 2,4–D and 2,4,5–T up to 72 hours after the initial application demonstrates that damage to the translocation system had not been great.

In the case of bean and marabu, Hay (10, 11) reported that the translocation of IAA and 2,4–D was greatly impaired within 8 to 24 hours after 2,4–D had been introduced into the plants. The variance between the results presented and those of Hay might be due to differences in the plant material or the growth substance concentration.

SUMMARY

The inhibition of mesquite root elongation was used to study the inhibitory activity, absorption and basipetal translocation of sublethal concentrations of 2,4–D and 2,4,5–T. The inhibitory activity of 2,4–D exceeded that of 2,4,5–T when these compounds were added to the root bathing medium. On the other hand, 2,4,5–T exhibited greater inhibitory activity than 2,4–D when applied to the cotyledons. Root inhibition was first apparent from four to six hours after cotyledonary applications. Ethanol, diesel fuel and acetone-nontoxic oil carriers did not readily penetrate mesquite cotyledons unless the epidermis was ruptured. Absorption of 2,4,5–T by cotyledons was greatest when acetone-nontoxic oil was used as carrier. The effectiveness of the carriers correlated with the surface area wetted by the drop applied. Repeated applications of 2,4–D and 2,4,5–T to the cotyledons caused little or no impairment of basipetal translocation 48 to 72 hours after initial application.

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The Fate of Long Chain Esters of 2,4–D in Plants[1]

D. James Morré and B. J. Rogers[2]

Esters of the chlorophenoxyacetic acids are generally considered to be more phytotoxic than the acid or acid salts (3, 12, 20). Hence the esters have found wide use in herbicidal formulations (12). Much research has been done on the mode of action of plant growth regulators, but the fate of neutral derivatives of active acids has received little attention, even though an acid side chain is one of the molecular requirements for growth substance activity (13).

The importance of 2,4–D esters and esters of other growth substances justified a study of the fate of 2,4–D esters in plants. Hagen, et al. (9) have shown with the butyl ester of 2,4–D and castor bean lipase that hydrolysis of these esters can be catalyzed by plant enzymes. The fate of long-chain esters of 2,4–D in plants has been studied by three assay procedures. A preliminary report has been presented (18).

Methods

Cucumber root inhibition test. Preliminary investigations revealed that standard assay methods for the detection of esterase activity were not sensitive enough to provide a measure of the hydrolysis of long-chain 2,4–D esters by plant extracts. It has been shown (19) that the octyl ester penetrates roots less readily than the free acid. This phenomenon was used as the basis for a sensitive bio-assay for quantitatively determining free 2,4–D acid in the presence of a low concentration of octyl ester. Cucumber seedlings *(Cucumis sativus* var. Marketer) were used as the test material. Cucumber seedlings germinated 48 to 52 hours, sized according to length of the primary root, were selected at random and grown for 12 hours on circles of filter paper containing 3 ml of the solution to be tested in a dark, constant temperature incubator at 24° ± 0.3° C. The humidity was maintained near 100 per cent by means of a plastic moisture chamber fitted inside the incubator. A concentration of 2.26×10^{-6} M (0.5 ppm, acid equivalent) of the octyl ester of 2,4–D (octyl 2,4–D) had no appreciable effect on the growth of cucumber roots during a 12-hour incubation period. The same concentration of 2,4–D acid resulted in a significant and reproducible inhibition (Figure 1). 2,4–D acid in the presence of higher concentrations of octyl 2,4–D could not be assayed unless diluted sufficiently so that less than 2.26×10^{-6} M (0.5 ppm acid equivalent) octyl 2,4–D remained.

The enzyme was extracted from cotyledons of dark-grown cucumber seedlings 72 to 96 hours of age which were ground in a pre-chilled mortar in a few ml of 0.1 M sodium chloride. More sodium chloride

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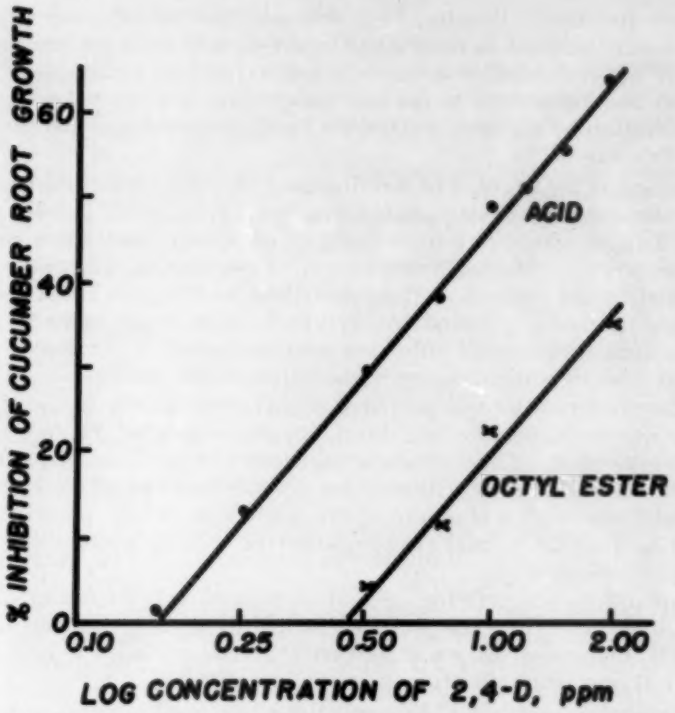

Figure 1. The effect of various acid equivalent concentrations of 2,4–D acid and the octyl ester (octyl 2,4–D) on the root growth of intact cucumber seedlings.

was added to give a ratio of 1 gm fresh weight to 4 ml of sodium chloride solution. The suspension was centrifuged to remove insoluble cellular material and the floating layer of fat which appeared during centrifugation was partially removed. The protein was precipitated from the clear supernatant fluid with sufficient solid ammonium sulfate to give 90 per cent saturation. After a second centrifugation, the pellet was resuspended in a minimum amount of 0.1 M sodium chloride. All preparatory steps were conducted at 4° C.

The octyl 2,4–D[3] substrate was prepared in 0.01 M sodium phosphate buffer, pH 7.0, to yield a final acid equivalent concentration of 2.25×10^{-6} M (0.5 ppm, a.e.). The solubility of the octyl 2,4–D in buffer was determined to be approximately 13.5×10^{-6} M (3.0 ppm, a.e.) by an adaptation of the general methods of Sobotka and Kahn (21). Enzyme preparations were added to provide a final concentration of 1 mg protein per ml of reaction mixture, unless otherwise indicated. Protein was estimated by a standard biuret method (8). The mixture was incubated for 12 hours at 24° C in a constant tem-

[3]Unformulated material furnished by Amchem Products, Inc., of Ambler, Pennsylvania.

perature incubator. Powdered egg albumin and boiled tissue preparations were utilized as controls. The 2,4–D acid released was measured by the cucumber root inhibition test. Increase in length of the primary root, expressed as per cent inhibition, was related to 2,4–D concentration by means of a standard curve prepared for each experiment (See Figure 1).

Chromogenic substrate. The development of color from the hydrolysis of the colorless *p*–nitrophenyl ester of 2,4–D (*p*–NP 2,4–D) provided a rapid sensitive test of the enzyme activity contained in an acetone powder obtained from cucumber cotyledons. The ester was prepared by the general method described by Huggins and Lapides (11) and partially purified by recrystallization from benzene and ether. Stock solutions of substrate were prepared in methanol and diluted with de-ionized water immediately before use.

An acetone powder was prepared from cucumber cotyledons dark-grown in vermiculite for five days. The finely ground, fat-free powder was stored at −32° C. Enzyme solutions were prepared by extraction with 0.1 M sodium chloride for several hours at 4° C. The undissolved material was removed by filtration. Crude protein was added to provide a final concentration of 0.1 mg protein per ml, unless otherwise stated.

Standard curves, relating optical density to concentration of *p*–nitrophenol (*p*–NP) were prepared according to Huggins and Lapides (11). Optical density was measured at 400 mμ with a Beckman Model B Spectrophotometer.

Bean epicotyl curvature. The curvature of kidney bean epicotyls was used as a measure of 2,4–D transport from the leaves. In this test, the time from treatment to the beginning of curvature is considered to be independent of concentration (7). The maximum curvature is used as a measure of the amount of 2,4–D absorbed and translocated; the rate of bending is also determined by dose (7).

The general procedures followed were explained in detail by Day (7). Red kidney bean seedlings were used when the primary leaves were almost fully expanded but before development of the terminal buds above the second node had occurred. With a micropipette, 10 μl of aqueous 2,4–D solution (5 μg) were applied to the upper surface of one leaf along the midrib 1 cm from the base of the leaf blade. Unformulated octyl 2,4–D and PGBE 2,4–D (propylene glycol butyl ether ester of 2,4–D) were applied as finely divided emulsions at the same acid-equivalent concentration. Ten to fifteen plants were selected for each chemical treatment at three different light intensities. The resulting curvatures were measured with a protractor.

In a second series of experiments, bean plants grown as above were placed in the dark at 25° C for 12 hours prior to the application of 2,4–D compounds. Plants were treated as rapidly as possible under very low light intensity to minimize production of carbohydrates, returned to the dark for an additional four hours, placed in the light (300 fc), and the curvatures then recorded.

RESULTS

Cucumber root inhibition test. The results of a typical experiment are presented in Table 1. The substrate of 2.26×10^{-6} M (0.5 ppm, a.e.) octyl 2,4–D had no significant effect on cucumber root growth. Reaction mixtures of crude protein from cucumber cotyledons plus

Table 1. Results of a cucumber root inhibition test for esterase activity. The reaction mixture consisted of 2.26×10^{-6} M (0.5 ppm, a.e.) of octyl 2,4–D in 0.01 M sodium phosphate buffer at pH 7 plus 1 mg/ml of total protein. Incubated 12 hours at 24°C prior to bio-assay.

Protein source	Root growth as per cent of buffer control
None	97
Cucumber cotyledons	70
Cucumber cotyledons (boiled)	105
Powdered egg albumin	105
Steapsin (pancreatic lipase)[a]	103
Wheat germ lipase[a]	100
Spinach acetone powder	73

[a]Nutritional Biochemicals Corp.

substrate, and spinach acetone powder plus substrate, respectively, resulted in 30 and 27 per cent inhibition of root growth. This corresponds to 100 per cent conversion of the octyl 2,4–D present to 2,4–D acid during the 12 hour incubation period. Boiled cotyledons and egg albumin, when added to the substrate, did not give results significantly different from the buffer controls. Commercial preparations of crude pancreatic lipase (steapsin) and wheat germ lipase did not result in measurable inhibition. These latter enzyme preparations catalyzed the hydrolysis of appreciable quantities of triacetin at pH 7 as measured by continuous titration of the acid released.

Extracts of pumpkin seeds and corn coleoptile, root tip sections, and embryo also catalyzed the hydrolysis of octyl 2,4–D. The enzyme activity was greatly reduced by the addition of 2×10^{-4} M copper sulfate (Table 2). This concentration of copper sulfate is known to

Table 2. The hydrolytic activity of crude extracts prepared from 20 mm. sections of corn coleoptile and root tips and excised corn embryos and the inhibitory effect of 2×10^{-4} M copper sulfate. Substrate 2.26×10^{-6} M (0.5 ppm, a.e.) octyl 2,4–D; incubated 12 hours at 24°C and assayed using cucumber root inhibition tests. Data are averages of 12 replicate samples.

Tissue extracted	Biuret protein mg/ml	2,4–D released (ppm)	
		Minus copper	Plus copper
None	0.00	0.00	0.00
Root	0.25	0.56	0.15
Coleoptile	1.75	0.60	0.15
Embryo	1.50	0.56	0.00
LSD 5%	—	0.54	NS

inhibit esterase activity (23). Total control growth was inhibited about 10 per cent by the concentration of copper remaining after a 2X dilution prior to bioassay.

The effect of enzyme concentration was determined with both crude cucumber cotyledon extracts and the spinach acetone powder. The data presented in Figures 2 and 3 are corrected for substrate and enzyme blanks.

Rate of hydrolysis as a function of substrate concentration is presented in Table 3, along with some indication of the error involved.

Table 3. Rate of hydrolysis of octyl 2,4–D as a function of substrate concentration. Crude cucumber protein concentration of 1 mg/ml; an average af 120 seedlings.

Substrate concentration Moles/liter x 10^{-6}[a]	Rate of hydrolysis Moles/liter x 10^{-6}[a] per hour
1.13	0.09 ±0.153
2.26	0.18 ±0.153
3.38	0.23 ±0.077
4.53	0.36 ±0.027
5.66	0.54 ±0.090
6.79	0.59 ±0.301
9.06	0.45 ±0.301

[a]2,4–D acid equivalent.

After addition of enzyme, the flasks were incubated for six hours and an aliquot was removed for assay. Substrate concentrations greater than 2.26×10^{-6} M could not be assayed by the usual procedure because of the possibility of response from unhydrolyzed ester. A preliminary assay was performed to approximate the extent of hydrolysis. The final assay was then carried out at a dilution to provide less than 2.26×10^{-6} M octyl 2,4–D per ml of test solution. Any kinetic treatment of the data is precluded due to the large variation resulting from low concentrations of 2,4–D acid and the dilution factor involved in assaying for 2,4–D acid in the presence of high ester concentrations. It is apparent, however, that the rate of hydrolysis is proportional to substrate concentration. After an additional six hours of incubation the 6.79×10^{-6} M substrate concentration resulted in root inhibition closely corresponding to complete conversion of the ester to the acid.

Chromogenic substrate. A number of substances were tested for hydrolytic activity. The results of an assay using 0.02 μM *p*–NP, 2,4–D are summarized in Table 4. The reaction rate was proportional to enzyme concentration and neither free *p*–nitrophenol nor methanol affected the course of the reaction at concentrations involved in the experiments. Reproducible inhibition by free 2,4–D acid could not be detected at substrate concentrations; however, 2,4–dichlorophenol resulted in a measurable inhibition at 3×10^{-4} M. Inhibition by 7×10^{-5} M copper sulfate was of the non-competitive type.

At a low substrate concentration (0.013 μM), the reaction followed first order reaction kinetics. At a substrate concentration of 0.66 μM the rate was linear with time until 85 per cent of the substrate was hydrolyzed, although the initial rate was less than that of low substrate concentrations (apparent substrate inhibition). Optimal

Table 4. The hydrolysis of *p*–NP 2,4–D under standard assay conditions in the presence of several proteins and amino acids at a concentration of 0.1 mg total protein or free amino acid per ml.

Protein or amino acid	Enzyme activity (units[a])
Cucumber acetone powder	6
Wheat germ lipase[b]	25
Steapsin[b]	34
Wheat germ lipase[b] (denatured)	0
Steapsin[b] (denatured)	0
Egg albumin	0.5
Histidine	0.25
Arginine	0

[a]The number of μM of *p*-nitrophenol liberated per minute with 0.1 mg of total protein in 3 ml of M/150 sodium phosphate buffer at pH 7.1 and 25° C.
[b]Nutritional Biochemicals Corp.

activity was obtained between pH 7.0 and 7.2 over a pH range from 6.0 to 7.8.

The substrate underwent non-enzymatic hydrolysis in M/150 buffer at pH 7.1, in methanol and in the crystalline state. It also was difficult to remove accompanying *p*–nitrophenol from the crystalline ester so that at the start of the reaction, 100 μM of substrate contained 30 μM of free *p*–nitrophenol and 30 μM of free 2,4–D. The substrate concentrations reported are based on the concentration of unhydrolyzed ester.

With a protein concentration of 0.1 mg per ml, hydrolytic activity of the cucumber acetone powder was determined with varied concen-

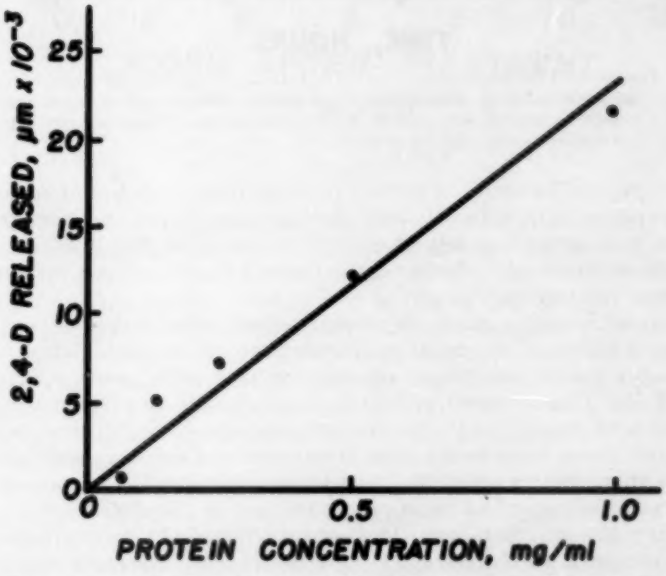

Figure 2. The relation between 2,4–D released from octyl 2,4–D, and concentration of protein prepared from cucumber cotyledons. Reaction mixture incubated 12 hours at 24°C prior to bio-assay.

trations of substrate in five experiments. From the double reciprocal Lineweaver and Burk (16) plot of velocity versus substrate concentration, the "apparent" Michaelis-Menten constant was determined, i.e., 2.8×10^{-6} M. In all cases inhibition was observed as the reaction approached the theoretical maximum velocity.

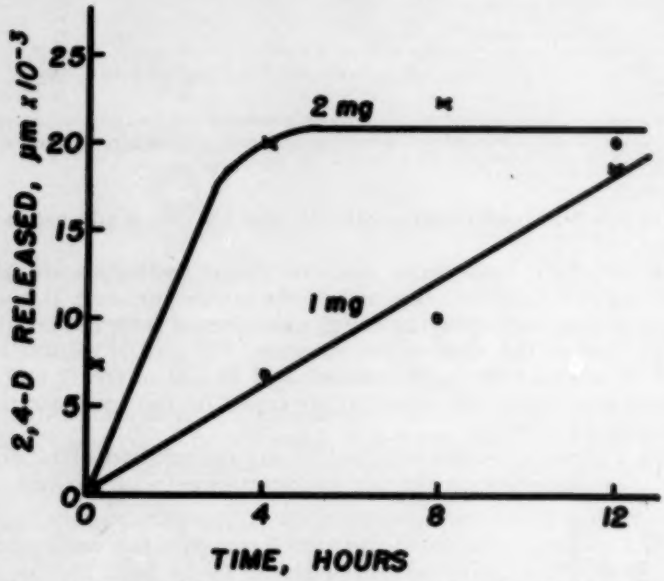

Figure 3. The hydrolysis of octyl 2,4–D as a function of time with two concentrations of protein prepared from a spinach acetone powder. Amount of 2,4–D acid released was measured by the cucumber root inhibition test.

Bean epicotyl bending. The degree of bending was found to be proportional to light intensity and the curvature from the ester-treated plants was somewhat less than that of the acid in all experiments (Figure 4). Formative effects on the treated leaves did not vary greatly between treatments.

If no allowance is made for time involved in initiation of curvature (7) the 2,4–D acid moved at an average rate of at least 2.7 cm/hr and the octyl 2,4–D moved at an average rate of at least 1.8 cm/hr. PGBE 2,4–D was tested at 300 fc, with results similar to those obtained with octyl 2,4–D. No further increase in curvature could be detected about nine hours after treatment and recovery was initiated about three hours later. No curvature from 2,4–D compounds was observed in destarched bean plants treated in the dark.

When low-starch plants (12 hours in the dark) were treated and then returned to the dark for four hours, the difference in time of initiation of curvature between acid and the ester was no longer evident (Figure 5). The response from all three compounds was parallel until about six hours after treatment.

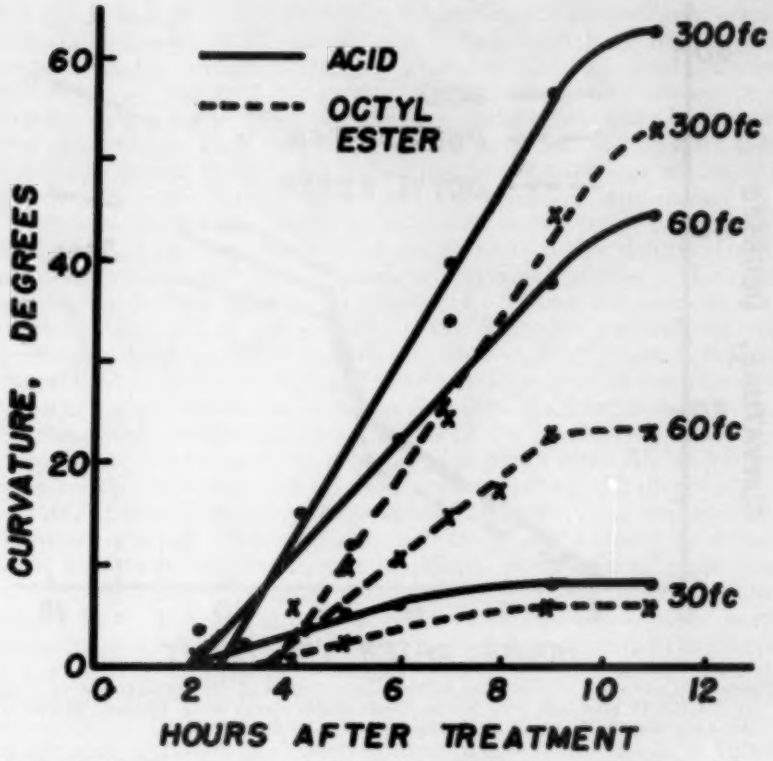

Figure 4. Curvature response of kidney bean seedlings to applications of 5 μg a.e. of 2,4–D and octyl 2,4–D at three light intensities.

DISCUSSION

Crude protein preparations from cucumber cotyledons and a spinach acetone powder apparently catalyzed the hydrolysis of octyl 2,4–D. During a 12 hour incubation period prior to assay, a 2.26×10^{-6} M solution of the ester was hydrolyzed nearly to completion, while the substrate alone did not exhibit a measurable amount of hydrolysis. The increased quantity of 2,4–D acid was the result of a specific protein effect, since when the protein was boiled there was either no effect or a slight enhancement of cucumber root growth. The effect of powdered egg albumin was similar to that of boiled cucumber cotyledons.

Digestive enzymes hydrolyze simple esters (1, 17). Commercial preparations of pancreatic lipase and wheat germ lipase were examined for activity; neither had any effect on cucumber root growth when subjected to the standard assay, although the same preparations actively hydrolyzed triacetin. Unfortunately, low conversion values of ester to acid did not always result in measurable inhibition of cucumber root growth and so might have escaped detection.

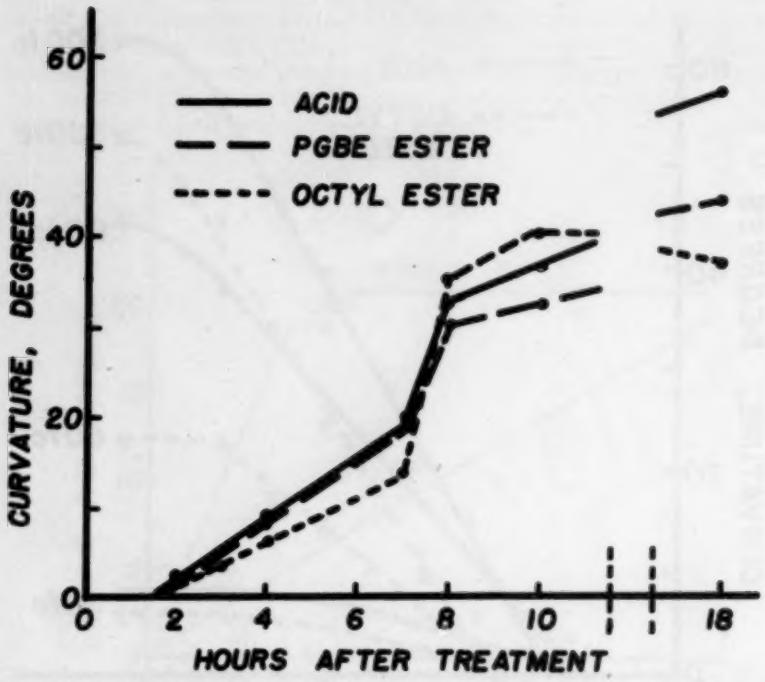

Figure 5. Curvature response of kidney bean seedlings to applications of 5 μg a.e. of 2,4–D acid and two esters. Destarched plants were treated, placed in the dark four hours, and returned to the light at zero time.

The increased inhibition of cucumber roots in the presence of protein preparations is interpreted as resulting from hydrolysis of the ester. The amount of product formed was proportional to the amount of protein added. The rate of hydrolysis was proportional to incubation time for a spinach acetone powder preparation. These data demonstrate the extremely low activity of the enzyme with respect to the 2,4–D octyl ester. The crude protein preparations used probably contained a small amount of natural substrate that could compete with the octyl ester for enzyme.

It was possible to show that the apparent hydrolytic activity was inhibited by 2×10^{-4} M copper sulfate. This concentration had been shown to inhibit esterases (23). Active hydrolysis of octyl 2,4–D apparently occurs also in corn roots, corn cotyledons, corn embryos, cucumber roots, and pumpkin seeds. The enzyme or enzymes involved probably are relatively group-specific for ester linkages; fractionation may reveal that several such enzymes are involved. The test, thus far, is limited to the octyl ester and apparently cannot include esters with lower oil/water partition coefficients.

The *p*–NP 2,4–D is hydrolyzed by a number of enzymes, including wheat germ lipase and animal lipase. Since lipases were not effective in catalyzing the hydrolysis of long-chain esters of 2,4–D, the *p*–NP

2,4–D did not serve as a specific assay for long-chain esterases as was originally intended. Histidine, which had been suggested as the active site for certain hydrolytic enzymes (2), also catalyzed the hydrolysis to a measurable extent. The chromogenic substrate provided a means for studying the kinetics of the 2,4–D ester hydrolysis and the effect of high concentrations of *p*–NP, 2,4–D suggests that failure to detect hydrolysis in a concentrated emulsion of the aliphatic esters may have been the result of substrate inhibition.

When seedling bean plants were treated with 5 μg of 2,4–D (acid equivalent) 1 cm from the base of one primary leaf, a definite lag in response was noted from the esters, which could be due to the time needed for hydrolysis prior to translocation, since the ester should penetrate faster than the acid (4, 5, 10). A similar experiment was performed by Leonard (14) with the methyl, hexyl, and octadecyl esters. Crafts (5) attributed the difference in results to the effect of size and weight of the molecule on mobility. In another test, Crafts (5) compared commercial formulations of the emulsifiable acid, isopropyl ester, PGBE ester, and the butoxyethanol ester. All molecules were rapidly absorbed and the four curves were almost identical.

The differences in magnitude of curvature in these studies may be more apparent than real, although the plants selected were uniform in outward appearance. It is difficult to explain why the rate of bending always terminated after about 9 hours, since the continued hydrolysis of the ester should eventually provide the same quantity of 2,4–D acid in all plants. Day (7) has suggested that there is a gradual equalization of concentration on all sides of the epicotyl, eventually resulting in apparent recovery. This might account for the fact that the ester did not cause the same magnitude of curvature as the acid, although the concentration of 2,4–D was the same in all cases. If the ester was not translocated out of the leaf, it must not have produced a measurable growth response, since the formative effects on the treated leaf were nearly the same in all cases.

The fact that translocation is proportional to light intensity for both the acid and the octyl 2,4–D response indicates a common dependence on carbohydrate transport, thus supporting a similar view taken by Linder et al. (15). The results are not easy to interpret and more direct evidence would be necessary to determine whether a free carboxyl group is required for transport as suggested by Thimann (22). The fact that the acid and the ester curves are nearly identical when destarched plants are treated in the dark and allowed to remain in the dark for an additional 4 hours suggests an *in vivo* hydrolysis. The rate of bending following initiation of curvature probably is related to changes in carbohydrate metabolism and does not directly affect interpretation of the results.

SUMMARY

1) Due to the difference in uptake, roots of cucumber seedlings were inhibited by 2,4–D acid over a narrow concentration range where the octyl ester had no effect on growth. Using this difference in response as the basis for a biological assay, certain of the criteria

for an enzyme catalyzed de-esterification of the octyl ester of 2,4–D were fulfilled. Although the rate of hydrolysis was low in all tissues studied, it appeared to be more than sufficient to account for complete hydrolysis in the intact plant.

2) A *p*–nitrophenyl ester of 2,4–D (*p*–NP 2,4–D) was synthesized and conditions were established by which the amount of *p*-nitrophenol liberated in the presence of a cucumber cotyledon acetone powder was proportional to hydrolytic activity. In contrast to octyl 2,4–D, this ester was hydrolyzed by lipases as well as the cucumber enzyme. The pH optimum in M/150 sodium phosphate buffer was between 7.0 and 7.2. An "apparent" K_m calculated for the standard assay conditions was in the order of 2.8×10^{-6} M. Excess substrate apparently inhibited the reaction.

3) Evidence for an *in vivo* hydrolysis of the octyl and propylene glycol butyl ether (PGBE) ester of 2,4–D was obtained by means of the curvature response of kidney bean epicotyls. A lag in initiation of curvature observed for the octyl and PGBE esters as compared to the acid was no longer evident under conditions where translocation was delayed until hydrolysis could occur.

ADDENDUM

Since this paper was submitted for publication a report appeared by Crafts citing evidence indicative of *in vivo* hydrolysis of the isopropyl ester of 2,4–D in intact barley leaves. WEEDS 8:19–25. 1960.

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The Effect of Certain Triazines on $C^{14}O_2$ Fixation in Red Kidney Beans

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SEVERAL triazines exhibit high phytotoxicity towards many species of higher plants and are being extensively studied as possible herbicides. Moreland et al. (3, 4) and Exer (1) reported that 2–chloro–4,6–bis(ethylamino)–*s*–triazine, simazine, inhibited the photochemical activity of isolated chloroplasts. Exer also presented evidence that the inhibited reaction involved the photochemical reduction of DPN, diphosphopyridine-nucleotide. Gast (2) has shown that in *Coleus Blunei* Benth. simazine blocked starch production in the light and that this effect was overcome by the addition of sucrose to starch-free leaves in the dark. These results indicated interference of photosynthetic CO_2 fixation by simazine. Roth (5) reported that simazine inhibited photosynthesis in Elodea but had a stimulating effect on respiration. In light of these findings a study of the effect of simazine and certain related triazines on the photosynthetic CO_2 fixation of red kidney bean was undertaken.

MATERIALS AND METHODS

Seeds of red kidney beans, *Phaseolus vulgaris* L. were lightly dusted with Arasan and planted in a flat of washed river sand. The flat was watered with Hoagland solution. When the first trifoliate leaf began to unfold (10 to 12 days) after sowing, plants of uniform size were selected and transferred to a tank of aerated Hoagland solution. After about 48 hours conditioning to the Hoagland solution new roots were beginning to develop. Replicate plants were placed in quart-size glass jars containing 950 ml of Hoagland solution. Appropriate amounts of herbicides dissolved in ethanol were added to the jars, resulting in a final ethanol concentration of 0.25% (v/v). The solutions were aerated. The control plants were treated identically including the addition of ethanol.

The plants were exposed to the herbicides for a specified time. One primary leaf from each treated plant was then excised at the base of the petiole under distilled water with a sharp razor blade and placed in a 4-dram vial, containing 5.0 ml of distilled water. The leaf outline was traced on graph paper, and the leaf area was determined by cutting out and weighing this area from the paper.

Four such leaves were transferred to a photosynthetic chamber. This was constructed from clear plastic with water filters on each side to absorb the heat from the light sources. The light was supplied by four 15-watt daylight fluorescent lamps on each side of the chamber giving a light intensity of approximately 1100 foot-candles from each side.

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Four leaves, three treated and a control, were conditioned for one hour by passing water-saturated air through the photosynthetic chamber under full light intensity. The chamber was sealed, and about 200 uc of $C^{14}O_2$ was generated by the slow addition of 10% lactic acid to a previously weighed amount (2 to 4 mg) of $BaC^{14}O_3$ (specific activity 66.8 uc/mg). The $BaCO_3$ was suspended in a few drops of distilled water in order to prevent spattering when the lactic acid was added.

The gas was circulated through the system with a diaphragm-type pump. A manifold with four openings permitted the simultaneous exposure of all leaves to the same amount of $C^{14}O_2$. The experiment was terminated after one hour by removing the leaves from the chamber and dropping them into 100 ml of boiling 80% ethanol. The leaves were homogenized in a Waring blendor, and the resultant suspension was filtered. The debris was washed with hot 80% ethanol, and the combined extracts and washings were made up to a final volume of 250 ml. One ml aliquots of the alcohol solutions were placed in cupped planchets, dried, and counted with a G. M. thin-end window counter. The radioactivity was corrected to 100 cm^2 leaf area, and these values obtained from treated leaves were compared with those of controls, giving "relative rates of photosynthesis".

These methods were used to conduct two types of studies; (1) the effect of various concentrations of simazine on $C^{14}O_2$ fixation after a 40 hour exposure to simazine and (2) a time-course study of $C^{14}O_2$ fixation of three triazine herbicides at 1 ppm.

RESULTS AND DISCUSSION

The effect of simazine at various concentration levels at a forty hour exposure to the herbicide, is presented in Figure 1. At a simazine concentration of 0.25 ppm, the relative rate of $C^{14}O_2$ fixation had decreased by about 30%. At 1 ppm and above, $C^{14}O_2$ fixation was almost completely blocked.

The effect of exposure time on $C^{14}O_2$ fixation was studied, using simazine and two related compounds; 2–chloro–4–ethylamino–6–diethylamino–*s*–triazine, trietazine (G–27901), and 2–methoxy–4,6–bis(ethylamino)–*s*–triazine, simetone (G–30044).[2] The plants were exposed to these herbicides for 1, 3, 6, 24, and 48 hours at a concentration of 1 ppm.

The data from the time-course study are given in Figure 2. There appeared to be an initial increase in the $C^{14}O_2$ fixation at the one hour exposure time for all three compounds. At the three hour exposure time, simazine inhibited the $C^{14}O_2$ fixation, simetone was similar to the control, the trietazine still appeared to stimulate $C^{14}O_2$ fixation. At the 6-, 24-, and 48-hour exposure times, all three compounds markedly inhibited $C^{14}O_2$ fixation.

It is quite evident from these data that the triazines drastically

[2]The authors wish to thank Clayton E. Bartley, Geigy Agricultural Chemicals, for samples of the triazines used in these experiments.

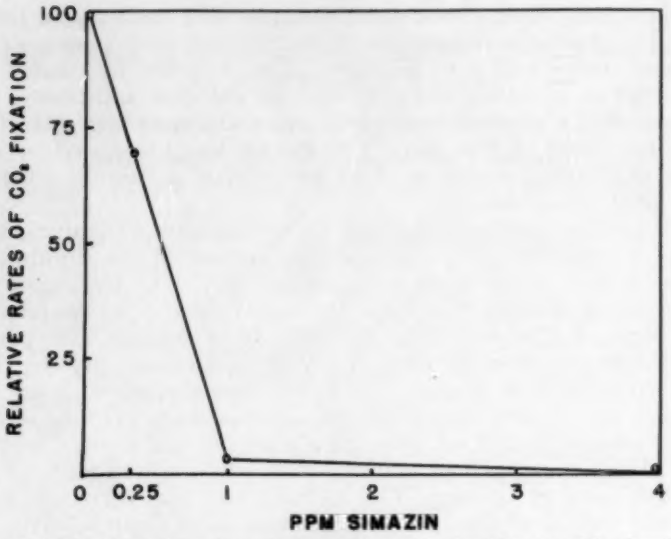

Figure 1. The effect of various concentrations of simazine on CO_2 fixation in red kidney bean after a 40 hour exposure to simazine.

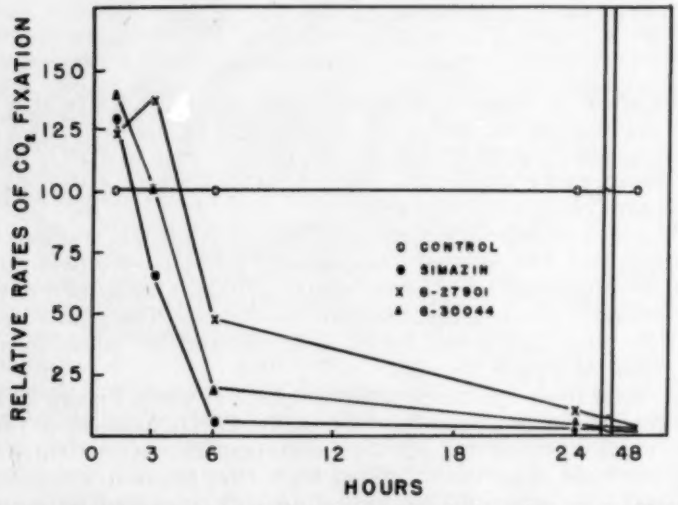

Figure 2. A time course study of the effect of 3 triazines, 1 ppm in Hoagland solution, on CO_2 fixation in red kidney bean.

inhibit CO_2 fixation in the light. The degree of inhibition increases with higher concentration of the herbicide and longer exposure time. This is undoubtedly an important factor in the phytotoxic

characteristics of these compounds. Additional work is in progress to study the effect of certain triazines on the metabolic pathway of $C^{14}O_2$ fixation.

SUMMARY

Simazine and related triazines drastically inhibit CO_2 fixation in the light. This is undoubtedly an important factor in the phytotoxic properties of these compounds.

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Biochemical Analyses, Germination, and Production of Black and Brown Seed of *Halogeton glomeratus*

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Halogeton glomeratus was first discovered in the United States growing in the vicinity of Wells, Nevada, in 1934–35. Since that time it has invaded Utah, Nevada, Wyoming, Montana, Colorado, Idaho, Oregon, and California. The total area it infests now exceeds 11 million acres.

Halogeton has been responsible for the death of numerous sheep in Nevada, Utah, and Idaho. While the sheep industry has not lost an excessive total number of sheep, individual raisers have had rather spectacular losses that have brought the halogeton problem to the attention of the public. Economic losses from deaths of sheep alone are no longer serious, because shepherds have learned to avoid ranges where halogeton constitutes a large percentage of the available forage. When losses do occur, however, the number of animals killed frequently ranges from 50 to 100 or even more.

The major economic loss due to halogeton today is the reduced grazing value of millions of acres of western range land where it has become a serious pest. This loss becomes increasingly serious as halogeton moves from strictly desert areas into the more productive range lands.

Halogeton produces both brown and black seeds (Figure 1). These adjectives have been applied because the two types appear to contrast in color as black and brown. The seeds designated black are actually a dark chocolate brown and somewhat smaller than the

Figure 1. Left to right: black seed in winged bracts; black seed with bracts removed; brown seed with short adherent bracts.

brown seeds. The five-winged papery sepals which surround the black seed give halogeton the appearance of being in flower during

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the late summer and early autumn. With the appearance of these winged sepals halogeton has entered the "bract" stage of growth. Since the term "bract" is more commonly used by those engaged in halogeton research, it will be used here in preference to the more botanically-correct "sepal". Upon drying, the bracts are easily removed, leaving the embryo encased in the integuments and ovary wall.

The brown seeds are lighter in color and the five bracts are short and adherent and do not develop the showy wings. The adherent bracts make the brown seeds appear somewhat larger than the black ones. The embryos of black seeds, however, are slightly larger than those of the brown. The dry weight of a black seed embryo averages 390 micrograms vs. 352 micrograms for the brown.

The role of the brown seeds in the reproduction of halogeton has been a matter of concern to workers engaged in halogeton research, particularly those conducting experimental work with herbicides and in closely-related fields of practical control. Black seeds germinate rapidly and establish readily whenever conditions permit. Several investigators (2, 5, 7) demonstrated that black seeds could germinate in solutions with pH values of 4 to 9, but they obtained optimum germination under slightly acid conditions. Black seeds placed on moist filter paper absorb water so rapidly that the ovary walls may rupture and expel the embryos within an hour. The embryos uncoil at once and frequently the resulting seedling can be planted within 9 or 10 hours.

Brown seeds, on the other hand, have rarely been observed to germinate under laboratory conditions. They swell only slightly in water and the embryos seem unable to penetrate the protective ovary walls. Upon excision and under strong light, brown embryos rarely uncoil in fewer than 48 hours. At this time a green pigment assumed to be chlorophyll appears in the cotyledons. The black embryos contain chlorophyll before emergence. Despite these differences, viability of seeds of both types stored in the laboratory for 1 year is about equal and extremely high.

The investigations reported here were conducted to determine biochemical differences between black and brown seeds, factors influencing production, and the possible role of the brown seeds in halogeton infestation.

METHODS AND MATERIALS

Analysis for Na, K, Ca, Mg. An analysis for Na, K, Ca, and Mg was run on embryos of brown and black seeds. The seeds were soaked in distilled water for 24 hours so that their embryos could be excised. Three grams of dried embryos of each type were digested separately in a 1:1 mixture of HNO_3 and $HClO_4$, and the mixture was evaporated to near dryness and brought to a final volume of 100 cc with distilled water. The analysis for Na, K, Ca, and Mg was run on a flame spectrophotometer (13).

Analysis for carbohydrates, protein, and oxalates. The procedures

for seed analysis were those developed for carbohydrates by Nelson (10) and Somogyi (11), for protein by Lowry et al. (8), and for oxalates by Miller and described by Dye (3). The analysis was conducted by using 150 seeds for carbohydrate determination and 30 for protein determination, thus eliminating the necessity of correcting for weight. Two-gram samples of each seed type were used in the oxalate determination.

Germination tests. Experiments to test the germination of normal brown seeds and excised embryos from brown seeds were conducted in the laboratory. These consisted principally of standard germination tests in which the seeds of excised embryos were placed on moist filter paper in petri dishes and held at 30 degrees C. for 12 days. The filter paper was moistened initially with a 0.05 percent solution of Panogen (2.2% methyl mercury dicyandiamide) to prevent growth of fungi. The excised embryos were placed under a fluorescent light suspended 2½ feet above the petri dishes.

Both brown and black seeds collected from 1953 through 1957 were placed at depths of 0, ¼, and ½ inches in desert soil in metal flats. Each flat contained 300 brown or 300 black seeds of halogeton evenly divided among the 3 depths used as treatments. The flats were placed outside the greenhouses on December 23, 1957, on the Utah State University campus, where they were subjected to normal winter fluctuations in moisture and temperature. The number of seedlings which germinated and became established was recorded daily for 2 months.

Photoperiod studies. Black seeds of halogeton were germinated in April in perlite which was moistened with a 1:4 dilution of Hoagland's nutrient solution (4). At the end of 2 weeks the plants were transplanted into soil in plastic quart containers and arranged on a well-lighted greenhouse bench. The normal day length was supplemented until midnight by light from two 200-watt bulbs. These bulbs were suspended at a height of 4 feet, but not directly above the test plants, because they were being used to provide a more intense light for an unrelated experiment. The test plants were then actually receiving indirect light of 60–400 fc at distances of 5 to 11 feet.

In late June, just as the day length began to shorten, the plants were divided into replications of 10 and treated as follows:

1. Normal day length plus supplemental lighting of 60–400 fc until midnight.
2. Normal day length only.
3. Absolute darkness for 3 days, then normal day length.

Duplicate set of plants were treated in the same manner but were also sprayed once with 20 ppm gibberellic acid.

In addition, four halogeton plants grown from brown seeds were treated as follows:

1. Normal day length.
2. Normal day length plus spraying with 20 ppm gibberellic acid.

No temperature controls were used. The plants, therefore, were grown under somewhat higher temperatures than would be encountered naturally.

RESULTS

Na, K, Ca, and Mg analysis. Two differences were noted in the inorganic contents of the embryos of the two seed types (Table 1). The brown embryos contained only one-fourth as much Ca as did the black. The ratio of the Na to K was the reverse of that found in

Table 1. Major mineral constituents of embryos of brown and black seeds of halogeton.

Seed type	Na ppm	K ppm	Ca ppm	Mg ppm
Brown embryos	1580	5870	620	3400
Black embryos	1290	5800	2400	3460

other halogeton tissues in that K was present in much greater quantities than Na. Other investigators (1, 3, 9) have found the quantities of Na to be greater than K in ratios ranging from 2:1 to more than 30:1, the ratios depending upon the tissue under consideration. None of the chemical differences observed in the embryos suggested a satisfactory explanation for physiological variation.

Carbohydrate analysis. Several differences of importance were found in the brown and black embryos upon analysis for reducing sugars, sucrose, and starch (Table 2). Because the bracts of the brown seeds

Table 2. Chemical analysis of brown and black seeds of halogeton for reducing sugars, sucrose, and starch. Each value is a mean based on 5 samples.

Seed type	Reducing sugars per seed μg	Ratio	Sucrose per seed μg	Ratio	Starch per seed μg	Ratio
Black	3.93	1.78	35.2	2.06	26.8	0.38
Brown	2.20	——	17.1	——	68.3	——

were necessarily included in the analyses, a sample of bracts was examined separately to determine whether carbohydrates were present. Because no starch or sugars were found, the bracts were considered principally cellulose, which would not interfere in biochemical analyses for carbohydrates.

Starch was the principal carbohydrate present in the brown seed. About 78 percent of the total carbohydrates in the brown seeds was found to be starch and 22 percent to be reducing sugars and sucrose. The reducing sugars constituted a relatively small portion of the total sugars present. Sucrose, on the other hand, was the principal reserve in black seeds. Nearly 60 percent of the total reserves was in

the form of sucrose or reducing sugars, and only 40 percent was found as starch. Again, reducing sugars constituted a relatively small portion of the total sugars initially available to the embryo.

Protein analysis. Protein analysis of the two seed types again revealed striking differences. Black seeds usually contained at least twice as much protein per seed as the brown. Black seeds contained about 165 micrograms of protein per seed, while the brown averaged 80 micrograms.

Oxalate analysis. Both types of seeds were low in oxalic acid. Some investigators have found as much as 8 percent, but the samples analyzed in this laboratory averaged 2 percent regardless of seed type.

Germination studies. The soil in the flats containing the black and brown seeds was thoroughly soaked by a heavy snow which fell and subsequently melted within 2 days after the flats were placed between the greenhouses. The precipitation was followed by several days in which the maximum temperature reached 45 degrees F.

The brown seeds in the flats did not germinate between December and July. The black seeds began germinating as soon as moisture was available and several seedlings were established within the first week. At the end of the first month, germination ranged from 11 to 29 percent and at the end of 2 months from 22 to 79 percent. Germination was highest in year-old seed and lowest in the 4-year seed from 1953. As indicated in Table 3, good germination was

Table 3. Germination of black seeds of halogeton buried at depths of 0, 1/4, and 1/2 inch in desert soil and exposed to normal winter temperatures beginning December 23, 1957.

Year seed collected[a]	Depth	Percent germination after	
		1 month	2 months
1953	surface	11	22
	1/4 inch	0	2
	1/2 inch	1	2
1955	surface	21	33
	1/4 inch	4	33
	1/2 inch	6	16
1956	surface	29	79
	1/4 inch	9	73
	1/2 inch	3	49
1957	surface	11	30
	1/4 inch	1	30
	1/2 inch	1	17

[a]Seed produced in 1954 had been improperly stored and was not viable.

obtained when seeds were placed on the surface or buried to a depth not exceeding 1/4 inch. Emergence was considerably lower when the seeds were planted at 1/2 inch.

Photoperiod studies.

The plants in nutrient solution directly under the lights which received a maximum intensity of 450 to 630 fc did not flower, even

though they were not harvested until late August. The light intensity received by the halogeton plants being specifically tested for photoperiodic responses ranged from 60–400 fc. All plants, regardless of treatment, were observed to begin flowering shortly after July 14. A light intensity in excess of 450 fc was necessary to prevent flower initiation under greenhouse conditions.

All plants on normal day length, regardless of any other treatment such as gibberellic acid or several long nights, proceeded to flower and produce both brown and black seeds.

All plants which remained near the supplemental light flowered, but produced only one or two brown seeds per leaf cluster (Figure 2.). In a normal life cycle, flowering would then have intensified,

Figure 2. Branch tip of halogeton which has produced only brown seeds for 4 months in response to normal day length plus supplemental lighting of 300 fc. Arrow indicates a mature brown seed.

resulting in the production of 3 to 6 black seeds per cluster. The plants continued to flower slowly and produce only brown seeds for 4 months until the termination of the experiment in late November.

In October, 10 plants in the group producing only brown seeds under supplemental illumination were placed in another section of the greenhouse where they received only normal day length. Flowering immediately intensified and within 2 weeks the plants were entirely covered with winged bracts containing black seeds (Figure 3). Immediately after black seed formation, the plants died. As

Figure 3. Winged bracts containing black seeds of halogeton. This plant had been producing brown seeds for 3 months. The picture was taken in late October, 15 days after supplemental lighting had been discontinued.

shown by Jansen and Cronin (6), the formation of black seed is the terminal function of halogeton and death follows, regardless of conditions favorable for continued growth.

In a subsequent experiment, plants which had produced only brown seed were removed for 1, 2, 3, 4, and 5 days to normal October day length and then replaced under the supplemental light. Plants exposed to 2 or more long nights produced black seeds, even though they were returned to a regime favoring only brown seed

production. The number of black seeds produced varied directly with the number of long nights, however. Even though fewer black seeds were produced after 2 or 3 long nights, the plants promptly died.

Gibberellic acid (14) was applied to study its effect on seed production and more specifically whether its use would alter the ratio of black and brown seeds. The use of this substance was without effect.

Mature halogeton plants grown from brown seed produced both brown and black seeds in the usual ratio (1 brown to 3 black) and were indistinguishable in appearance from plants arising from black seeds. The 4 plants used represented a total survival from among 300 excised embryos originally germinated. Usually no difficulties are encountered in establishment if the seedlings survive through 2-leaf stage, but mortality before this stage was exceptionally high.

DISCUSSION

Although several distinct biochemical differences between black and brown seeds of halogeton have been shown, none explained the extreme lack of vigor in the brown type. The carbohydrate reserve was sufficient in the brown and the lower protein content was not considered inadequate for the needs of the embryo. The black seed would be able to capitalize upon favorable growing conditions because of its high sucrose content. This would possibly explain its ability to germinate under extremely cold conditions during the winter. Conversely, the brown type should enjoy equal success in germination under optimum temperatures which would be conducive to catabolism of its superior carbohydrate reserves. This, as has been shown, does not occur.

The brown seeds have been considered immature black seeds, but this theory is invalid because brown seed is produced first. Under controlled light conditions, it is the only type produced and is physiologically mature in every respect. It is assumed here, that by immature, reference is made to a seed which would have been physiologically different had it been allowed to terminate growth in a normal manner. Brown seed must be considered mature, therefore, since continued growth of the plant does not alter the physiological or biochemical composition of the seed.

It is possible that the brown seed is dormant and reaches its maximum ability to germinate one to several years after formation. Since the majority of the black seeds germinate the first year, brown seed dormancy would be a natural method of insuring the continuity of the species. Laboratory samples of brown seeds stored at room temperatures tend to lose viability almost as rapidly as black seeds. The question of longevity and viability under natural conditions has been studied by Tisdale and Zappetini (12) and is currently being investigated through a 10-year seed burial study under the direction of U. S. Department of Agriculture research personnel at Pullman, Washington. The study was initiated in 1957 and is

being conducted concurrently in Idaho, Nevada, Utah, Washington, and Wyoming. Both black and brown seeds were buried in plastic screen bags at 0, 1, 3, and 6 inches in typical low-precipitation areas in the states mentioned. First year results reported by Robocker and Kerr[2] showed that 100 percent of the black seeds germinated during the first year, regardless of site or depth of burial (with the exception of Idaho where 1 sound, ungerminated seed was found and subsequently germinated in the laboratory). Almost none of the brown seed had germinated except in Washington where 60 percent germination occurred in the surface treatment. Viability of brown seed at the end of the first year ranged from 100 percent in all buried samples in Utah to 0 percent in all buried samples in Washington. The study should provide some much needed data on the longevity and potential danger of the brown seeds.

The production of brown seeds in the greenhouse occurs on long days if the long day consists of a short day plus indirect supplemental lighting, the intensity of which varies from 60 to 400 fc. A short day augmented by strong supplemental lighting of at least 450 fc inhibits flower formation in the greenhouse. On the desert it would appear that brown seed production represents a response to a critical photoperiod beginning in mid-July, during which brown seeds are then produced for several weeks. By mid-August a second critical photoperiod is reached after which only black seeds are formed. If the plant is producing only brown seed it lives indefinitely. Death follows black seed production. Plants which germinate in August in the greenhouse or on the desert have been observed to produce only black seeds.

The change from brown to black seed production is abrupt. Nevertheless, a few seeds which appear to be intermediate have been observed. Several of these were noted during first year germination tests with brown seeds from the 10-year burial trials and a considerable number were recovered from the plants used in the greenhouse photoperiod experiment. Upon visual observation, these seeds appeared to be typical brown types. However, a true brown embryo is pale yellow throughout when excised, while the true black embryo consists of green cotyledons and a white hypocotyl. The embryo of the intermediate seed has reddish cotyledons and a white hypocotyl. In emergence and uncoiling it is less vigorous than the black seed, but much more active than the brown.

SUMMARY AND CONCLUSIONS

1. Black seeds stored the major portion of their reserves as sucrose, while the reserves of brown seeds occurred principally as starch. Brown seeds had a higher total carbohydrate reserve. Black seeds contained twice as much protein.

[2]Robocker, W. C., and Kerr, H. D. Report, presented at the Range Weed Research Meeting, Salt Lake City, Utah, March 16, 1959.

2. Black seeds germinated rapidly. Brown seeds were viable, but germinated very poorly. These studies suggest that halogeton is very largely established from black seed.
3. The role of the brown seeds remains obscure. One possible function would be a delayed germination period which would extend the viability of seed over several years. Laboratory observations have shown that brown seed loses its viability rapidly after 4 years, but that its ability to germinate did not increase during that time. A 10-year burial study now in progress under field conditions may supply additional information on the role of the brown seeds in the establishment of halogeton.
4. Brown seeds are formed as a first photoperiodic response to the shortening days of midsummer. The setting of black seeds in the greenhouse could be prevented by supplementing normal day length until midnight with light of 60–400 fc intensity.
5. Plants artificially induced to produce only brown seeds formed additional flowers that gave rise to black seeds when placed on short days. The plants died immediately after the production of black seeds.
6. Halogeton germinated after August 15 and grown continuously on normal daylight formed only black seeds.
7. Intermediate type seeds were observed.

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BRIEF PAPERS

Iso-octyl Ester of 2,4,5–T in Hardwood Control

CARTER B. GIBBS[1]

DAVIS and Duke (2) in 1955 reported successful use of a 36 lb ahg solution of the propylene glycol butyl ether ester of 2,4,5–T applied by means of a tree injector.[2] This tool is essentially a modification of the Cornell tool described by Cope and Spaeth (3) in 1931, and subsequently tested with ammonium sulfamate (1, 5) and 2,4,5–T (4, 6, 7).

Since the iso-octyl ester of 2,4,5–T is generally available at lower cost than the previously tested propylene glycol butyl ether ester, a study to assess its effectiveness was installed in March 1957 by the Nacogdoches Research Center in cooperation with the Sam Houston National Forest. Each of four concentrations of the chemical, 40, 20, 13.3, and 8 lb ahg in diesel oil, was applied to 30 sweetgums (*Liquidambar styraciflua* L.) and 30 oaks (*Quercus stellata* Wangenh., *Q. marilandica* Muenchh., and *Q. falcata* Michx.). Trees were of two diameter classes, 0.6–3.5 inches and 3.6–6.5 inches. A total of 480 trees were thus injected—all in circumbasal wounds, one per inch of estimated tree diameter.

Since an aim of the study was to examine the effectiveness of various concentrations under field conditions, no special measures were taken to assure exactly uniform applications of the silvicide. Each treated tree was inspected for crown condition and sprouting in May and October 1957 and in November 1958.

At the first inspection, three months after treatment, crown damage was visible only on trees treated with the higher concentrations. By the end of the first growing season the most effective treatment, 40 lb ahg, had top-killed 100 percent of the small trees and 93 percent of the large trees. Top-kill was slower on sweetgum than on oaks. No treated trees sprouted during the first growing season.

Results at the end of the second growing season are shown in Table 1 as percents corresponding to the mean angle of equal information (arcsin $\sqrt{\%}$ transformation). The difference between 100 percent top-kill for 40 lb ahg and 75 percent for 20 lb ahg was highly significant. The 64 percent top-kill with 13.3 lb ahg was significantly more effective than 33 percent with the 8 lb ahg. Differences in complete kill were similarly significant. Differences in both top-kill and complete kill between oaks and sweetgum were significant, an indication that sweetgum was harder to kill than the oak species tested.

There were no significant differences in either top-kill or complete

[1]Nacogdoches Research Center, maintained at Nacogdoches, Texas, cooperatively by Stephen F. Austin State College and Southern Forest Experiment Station, Forest Service, U. S. Department of Agriculture.

[2]Manufactured by Reuel Little, Madill, Oklahoma.

Table 1. Top-kill and complete kill two growing seasons after injection with 2,4,5–T.

Concentration (lb ahg)	Trees top-killed, pct			Trees killed, no sprouts, pct		
	Oak	Sweetgum	Both	Oak	Sweetgum	Both
40	100	100	100	100	100	100
20	92	54	75	82	50	67
13.3	69	55	64	64	54	59
8	39	27	33	35	23	29

kill between the two tree size-classes, though the total percentage of trees sprouting (including those with live tops) was significantly greater for the smaller size class.

The effectiveness of iso-octyl ester of 2,4,5–T when injected into oaks and gums in east Texas means that choice of a low-volatile ester of 2,4,5–T can be based on relative costs of the chemical. Where only oaks are to be controlled, the 20 lb ahg concentration of the iso-octyl ester may be satisfactory. Where sweetgum or other species with similar resistance to silvicides are to be treated, a higher concentration will be required.

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An Experimental Plot Sprayer

K. F. Best and A. Wenhardt[1]

Introduction

Good plot sprayers are essential in weed control investigations where chemicals are to be applied uniformly and in specified quantities. Derscheid[2] described such a machine in 1952 and this machine, or similar models, have been used quite widely. The purpose of this paper is to report on a sprayer which was built as an improvement on Derscheid's machine. A number of these modified sprayers have been built at Swift Current and used successfully by other weed workers in Canada.

Procedure

The sprayer is shown in Figure 1. Its similarity to Derscheid's machine is readily seen. A single rear castor wheel has been added. This serves to keep the machine in an upright position at all times. In addition to ensuring a level plane of operation, this castor wheel

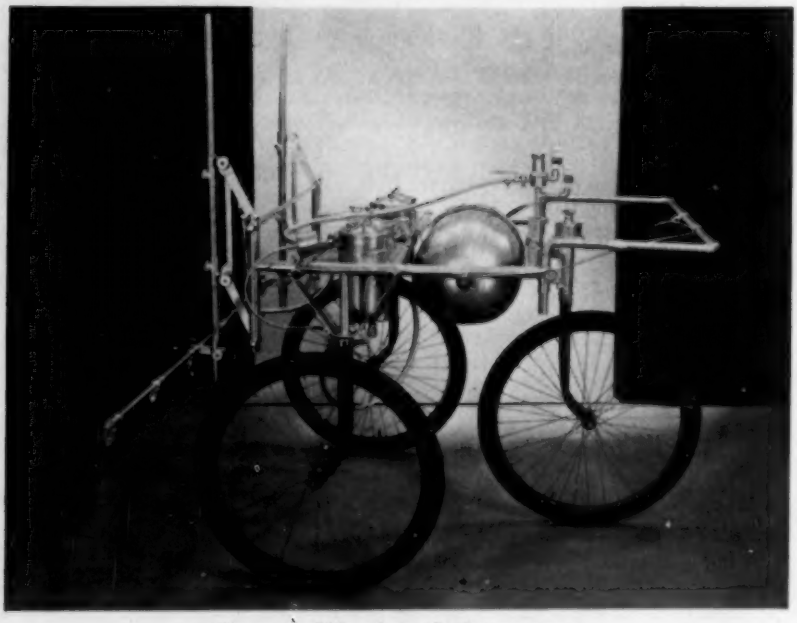

Figure 1. Side view of plot sprayer.

[1]Research Officers, Experimental Farm, Canada Department of Agriculture, Swift Current, Saskatchewan.

[2]Derscheid, L. A. Weeds. 1:329–337. 1952.

also assists in maneuverability. A manual control incorporated into the castor wheel mounting permits easy swivel tension adjustment. The wheel can be locked in a forward position or allowed to pivot under different tensions.

A positive boom height adjustment has also been incorporated. The boom is so mounted that the nozzles are perpendicular to the ground surface regardless of height. Variations between 12 and 72 inches are made possible by readily accessible adjustments on the boom.

The compressed air tank pressure gauge, the air pressure regulator gauge, and the spray control valve are mounted directly in front of the operator. An additional pressure gauge has been mounted on the boom to indicate the pressure under which the solution is applied. The overall weight of the machine is approximately 140 pounds.

The cost of materials for the construction of this machine was $250.00 and approximately 50 hours of labor were required. Complete drawings are available from the Superintendent, Experimental Farm, Swift Current, Saskatchewan.

Notes on the Distribution of the Russian Thistle (*Salsola kali* L. var. *tenuifolia*) in Mexico

PAULINO ROJAS-M. and M. ROJAS-GARCIDUEÑAS[1]

THE Russian thistle (*Salsola kali* L. var. *tenuifolia* Tausch) called in some parts of México "saladilla" is an important weed in the dry regions of the United States. It was introduced in 1873 or 1874 to South Dakota and from there migrated to the West and Southwest, reaching California between 1892 and 1895 (6), where it constitutes a serious problem.

The fast invasion by this plant of the United States semiarid lands would be expected to continue into the Northern States of México in which a semiarid climate is found too. However, it was not reported by Muller, either in Nuevo León (4) or in Coahuila (5), as late as 1947; also, it is not reported by Leseur who studied the northern part of Chihuahua (3).

It seems that *S. Kali* did not exist abundantly on the other side of the border because Cory and Parks in 1937 (1) mention it in their Catalogue of the Flora of Texas but Reeves and Bain do not include it in 1947 as a component of the flora of South Central Texas (7).

Indeed some individuals were present in México, but probably in a very scanty number; thus Johnston (2) reports that Endlich collected the species between Mapimí and Ojuelos, Durango (Figure 1); however the plant was not found by Johnston, who collected in the States of Chihuahua, Coahuila and Northern Durango and Zacatecas. It is interesting to note that the plant was seen by only one worker rather far south from the border and none was found in the northern parts of México.

In recent papers by Rzedowski (8, 9) the plant is not mentioned either; however, in a personal communication it was indicated that this author collected *Salsola* both in Zacatecas (12 Kms. NE from Concepción del Oro) and San Luis Potosí (10 Kms. S from El Salado). In 1957, Valdés[2] collected in various regions of Northern México and found the plant around Torreón, Coahuila (Figure 1). Again, the species was found far from the border and apparently still scarce.

In October 1958, the senior author collected samples of *S. kali*, West from Monterrey, Nuevo León (Km. 333 on the highway to Saltillo) and he was told that the weed was established south of Saltillo in potato fields. In the summer of 1959 collected in the central and northeast part of México and observed *Salsola* in the following points (Figure 1):

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[1]Professors of Botany. Depto. de Parasitología y Botánica. Instituto Teonológico de Monterrey, México.

[2]Valdés, J. 1957. Contribución al estudio de la Vegetación y la flora en algunos puntos del Norte de México. Tesis. Universidad Nal. Autón. de México Fac. de Ciencias. México.

1. Town of Durango and surroundings.
2. From the Km. 231 of the Durango-Torreón highway till this last town.
3. In several points of the highway from Torreón to Saltillo, specially around Paila.
4. Saltillo and surroundings.
5. Monterrey and surroundings.
6. Km. 570 of the Central Highway (Piedras Negras-México) south from Matehuala.
7. On the highway from Monterrey to Matamoros in the outskirts of Reynosa, west till Matamoros.
8. Town of Matamoros and surroundings.

The plant was not seen in the region where Endlich collected it. Neither was it found between Monterrey and Reynosa, nor south from Matamoros. In some localities the Russian thistle was very abundant.

From these data it seems that *Salsola kali* var. *tenuifolia* migrated to the northeastern and northcentral part of México from Texas. Its early presence in Durango before being reported by any author except Endlich suggested that possibly it migrated through Chihuahua, although it has not been reported in this State. Also, it is possible that it has come through northern Coahuila, but no recent data on this region are available.

The Russian thistle is clearly of recent migration; it is little known by agriculturists and they state it was not present until recent times. The fast dispersion of *Salsola* to the west and southwest contrasts with the slow invasion to the south, perhaps because the dominant winds in the area of introduction come from the northeast or else, due to the recent development of communications in the northern part of México.

Now, however, *Salsola kali* var. *tenuifolia* is established in many localities of México and it will be one weed more and a difficult one to fight against.

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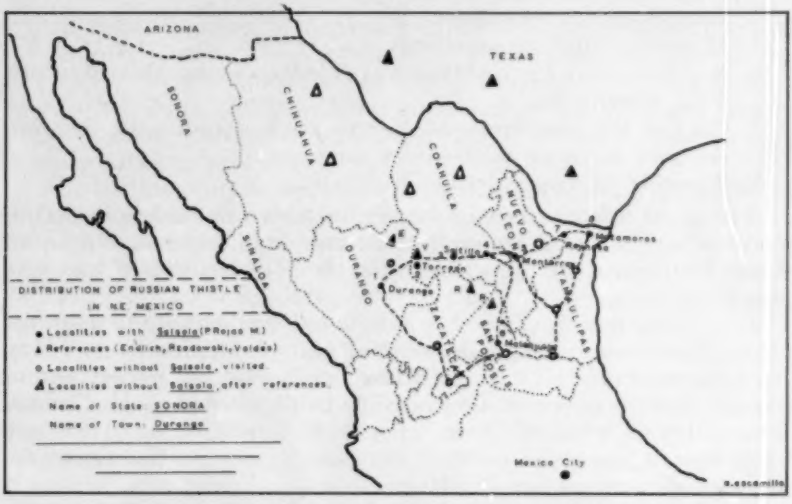

Figure 1. Map of México showing the distribution of *Salsola kali* according to trips and references. A new notice indicates a small colony of the plant was found by E. Hernández-X., close to México City.

Minutes of the Business Meeting and Committee Reports, Weed Society of America Meeting, February 22, 23, 24, and 25, 1960

Minutes

The third General Business Meeting of the Weed Society of America (WSA) was held at the Cosmopolitan Hotel, Denver, Colorado, 3:45 to 5:45 p.m., February 23, 1960. The meeting was called to order by A. S. Crafts, President of the Society. He outlined the significant accomplishments of the Society during the past 2 years and cited the excellent progress that had been made by the Constitution and By-laws Committee in revising the Constitution of the Society and stated that the new Constitution had been approved by an overwhelming majority of the membership.

Dr. Crafts indicated that the Executive Committee and members of the Society were especially pleased with the progress being made by the Terminology Committee in coordinating the standardization of terminology used in the field of weed control.

The President indicated that WSA was especially pleased to announce that the application of the National Weed Committee of Canada for membership in the Society had been overwhelmingly approved. He also indicated that a committee had been appointed to encourage an organization for Latin American weed workers.

Dr. Crafts expressed his appreciation for the excellent work of the various Standing and Special Committees and especially for the full cooperation that he had received from the membership of the Society. He emphasized that the Society exists to serve the needs of the members and that the ideas and suggestions of the members should be continuously solicited in an effort to improve the Society.

The President then requested that the minutes of the second Business Meeting of the Society be reviewed. W. C. Shaw, Secretary, indicated that the minutes of the second Business Meeting of the Society, January 13, 14, and 15, 1958 were published in Weeds 6: 331–341, 1958 and moved that the minutes be approved as published. The motion was seconded and passed.

W. C. Jacob, Treasurer-Business Manager, then reviewed in detail the financial status of the Society. The full report is included in these minutes.

Dr. Crafts indicated that the fourth meeting of the Weed Society of America would be held December 11, 12, 13, and 14, 1961 at the Sheraton-Jefferson Hotel, St. Louis, Missouri, with the North Central Weed Control Conference (NCWCC) serving as host conference.

The following Standing and Special Committee reports were presented by the chairman or an acting chairman of each committee. Following the presentation of each report, the chairman moved the adoption of his respective report. Each motion was duly seconded and each report was accepted by a majority vote of the members of the Society attending the meeting.

Respectfully submitted
W. C. Shaw, *Secretary*

STANDING COMMITTEE REPORTS

FINANCIAL REPORT, WEED SOCIETY OF AMERICA, DEC. 1, 1958–NOV. 30, 1959

Balance on hand Dec. 1, 1958			$9,728.96
RECEIPTS			
Memberships and subscriptions			
Volume 1	69.25		
Volume 2	68.00		
Volume 3	80.00		
Volume 4	114.00		
Volume 5	130.00		
Volume 6	179.84		
Volume 7	4,588.65		
Volume 8	2,418.00		
Volume 9	54.00		
Volume 10	6.00		
Total		7,707.74	
Reprints			
Volume 6	130.30		
Volume 7	1,211.42		
Total		1,341.72	
Advertising—Volume 7		1,000.00	
Sustaining Memberships 1959		3,100.00	
Abstracts			
1956	4.00		
1958	8.00		
Total		12.00	
Total			13,161.46
Total receipts			22,890.42

EXPENDITURES			
General Operating Expenses			
Office Supplies	104.23		
Mimeographing	50.46		
Mailing list and labels	37.90		
Postage and communications	647.40		
Secretarial labor			
for Bus. Mgr.	1,200.00		
for Editor	500.00		
Honoraria to Editor and Bus. Mgr.			
1958	1,000.00		
1959	1,000.00		
Bank expense	6.05		
Travel expense	135.41		
Total		4,681.45	
Publications			
WEEDS	8,063.22		
Reprints	1,096.12		
Refunds	11.50		
Total		9,170.84	
Total expenditures			$13,852.29
BALANCE ON HAND DEC. 1, 1959			9,038.13

WALTER C. JACOB
Treasurer-Business Manager

EDITORIAL COMMITTEE

Papers received from former editor, K. P. Buchholtz

	No.	Published since
Papers ready for final editing for printer	15	15
Reviewed by Editorial Committee, to be reviewed by editor and returned to authors	18	15
Paper in authors' hands after editorial review	9	2
Totals	42	32

Of the unpublished papers in the last two groups, one was rejected.

Four numbers of WEEDS were sent to the printer:

Vol. 7, No. 2	13 papers,	98 pages,	122 pages total
Vol. 7, No. 3	16 papers,	109 pages,	126 pages total
Vol. 7, No. 4	18 papers,	140 pages,	174 pages total
Vol. 8, No. 1	17 papers,	138 pages,	156 pages total

Vol. 7 contained 58 pages more than Vol. 6. Vol. 8, No. 2, in press, will contain about 160 pages, so we are now printing a consistently larger publication than in the past.

Papers received Dec. 1, 1958, to Dec. 1, 1959, total	80
Of these, on Dec. 1, 1959:	
Papers sent to printer	32
In hands of Editor, ready for printing	22
In hands of Editorial Committee	6
Returned by reviewers, awaiting editorial review	7
In hands of authors to be returned for final editing	6
Rejected	5
Combined with another paper by same author	2

O. C. LEE
R. A. PETERS
W. C. ROBOCKER
E. G. RODGERS
C. J. WILLARD, *Chairman*
Editor, WEEDS

EDUCATION COMMITTEE

Two reports prepared by a previous Education Committee will be brought up to date and reproduced. These reports deal with desirable courses for college students majoring in weed control and the course offerings in weed control in various institutions. Copies of these reports will be supplied workers engaged in academic activities in each state. Additional copies will be available upon request from Dr. Walter Jacob, Business Manager, University of Illinois, WSA, University of Illinois, Urbana, Illinois.

The Committee is developing a small folder describing the activities and opportunities in the field of weed control. The folder is intended for distribution to students in institutions of higher learning so that they may be aware of the possibilities for service in this field. The Committee believes that a determined effort should be made to inform the public of the importance of weed control. The opportunities for service in the field of weed control should be publicized so that a more highly trained, and a more specifically trained, personnel will become available for work on weed control and for the study of weeds.

C. L. FOY
W. R. FURTICK
L. J. KING
C. J. WILLARD
K. P. BUCHHOLTZ, *Chairman*

FINANCE COMMITTEE

The Finance Committee of the Weed Society of America recommends that $5,000.00 be invested in Government bonds. Although the interest rate is not as high as might be obtained elsewhere, the invested money would be readily available on short notice and would draw a reasonable profit without excessive risk.

S. M. RALEIGH
E. G. RODGERS
F. L. TIMMONS
W. S. VAN SCOIK
F. W. SLIFE, *Chairman*

LEGISLATIVE COMMITTEE

The Legislative Committee has reviewed a number of proposed resolutions concerning regulatory problems associated with the field of weed control. These resolutions were duly considered and referred either to the Resolutions Committee or the Executive Committee of the Society.

The Committee cooperated with the Chairman of Section IX, Regulatory Aspects of Weed Control, in developing the Regulatory Section Program for this meeting.

The Legislative Committee recommends that the members of the Society actively work to accomplish the objectives of resolutions which are adopted by the Society.

W. S. BALL
E. A. EPPS, JR.
T. J. MUZIK
J. C. WARD
JACK DREESSEN, *Chairman*

NOMINATING COMMITTEE

In the absence of S. N. Fertig, Chairman of the committee, A. S. Crafts made the Nominating Committee report. He indicated that the Nominating Committee had nominated 2 candidates for the office of President, 2 candidates for the office of Vice-President, and 3 candidates for the office of Secretary, and that the following officers had been elected by a mail ballot distributed to the membership of the Society:

K. P. Buchholtz, *President*
W. C. Shaw, *Vice-President*
F. W. Slife, *Secretary*

Dr. Crafts expressed his appreciation for the excellent work of the Nominating Committee and his regret that S. N. Fertig could not attend the meeting to present the report.

L. L. DANIELSON
B. E. DAY
B. H. GRIGSBY
G. C. KLINGMAN
S. N. FERTIG, *Chairman*

PROGRAM COMMITTEE

The Program Committee wishes to emphasize that the future success and growth of the Society will depend on the quality of WSA programs. The committee has made a special effort to solicit the suggestions of members of the Society for ways and means of improving Society programs. The 1960 WSA program, except for the General Session and Banquet Program, was developed almost entirely from volunteer contributions. This trend is extremely encouraging and indicates the contributions that members of the Society are making to its technical program.

The Chairman expressed his thanks to members of the committee for their assistance and excellent cooperation in developing the program. Special appreciation was expressed to the Sectional Program Chairmen for their contributions. The committee also wishes to acknowledge the excellent cooperation of the Local Arrangements Committee in providing the necessary facilities for an efficiently conducted program and to thank Jack Dreessen of the National Agricultural Chemicals Association for his assistance in arranging for the printing and distribution of the official program.

K. P. BUCHHOLTZ
W. A. HARVEY
D. L. KLINGMAN
G. F. WARREN
W. C. SHAW, *Chairman*

PUBLIC RELATIONS COMMITTEE

Since the last meeting of the Society, several letters from agricultural publications have been answered to provide information about activities of the Society.

Two general news releases announcing this, the third meeting, were mailed to newspapers, magazines and journals throughout the United States and to all newspapers in the state of Colorado.

One additional release was mailed to selected publications and radio stations to further publicize the meeting.

Since the meeting began the Committee has been in contact with THE DENVER POST and the ROCKY MOUNTAIN NEWS to supply information for news stories in these papers. Also the Farm Directors of two local radio stations have been contacted and a number of recordings will be broadcast to farm audiences this week.

The committee recommends that for future meetings papers be made available as far in advance of the meetings as possible to enable the committee to obtain wider coverage in newspapers and other media. This is particularly important in respect to papers to be presented at general sessions of the Society.

W. C. JACOB
LEONARD LETT
W. C. ROBOCKER
JACK DREESSEN, *Chairman*

TERMINOLOGY COMMITTEE

A brief description of the objectives, operating procedures, and accomplishments of the committee was presented. Two subcommittees which had been previously appointed functioned during the past 2 years in the development of terminology used in the field of weed control. These subcommittees are:

Subcommittee on Standardization of Abbreviations, Terms, and Definitions Used in Weed Control. K. P. Buchholtz, R. D. Sweet, F. L. Timmons, R. Behrens, Chairman

Subcommittee on Standardization of Common and Botanical Names of Weeds. L. C. Erickson, W. R. Furtick, L. G. Holm, M. M. Schreiber, R. A. Darrow, Chairman.

Both of these subcommittees have made excellent progress in developing standardized terminology in their areas of responsibility.

The Terminology Committee also made a special effort to enhance the acceptance of common names or abbreviations for new herbicides. The Chairman indicated that the cooperation which had been received from the entire chemical industry in sponsoring common names for herbicides was gratifying and that an intensive effort was being made to reduce the amount of time between discovery of new herbicides and the development of common names for such chemicals. The committee devoted considerable effort to making a survey of names for the discipline of weed control. More than 40 suggested

names for the discipline of weed control have been submitted, and the committee is in the process of reducing the proposed names to the 5 most desirable terms. The committee will continue to keep the Society membership advised of its progress in recommending a name for the discipline of weed control. W. C. Shaw indicated that a special effort will be made to revise the Terminology Committee Report at least annually. A detailed Terminology Committee Report was submitted for publication* in WEEDS.

The Chairman expressed his thanks to the members of the committee and especially to all members of both subcommittees for their assistance and excellent cooperation in developing the revised Terminology Committee Report. He also expressed his appreciation for the excellent cooperation that the committee had received from the industry in improving herbicide nomenclature.

Following presentation of the report, Lawrence Southwick of the Dow Chemical Company indicated that his Company had presented a proposal to the Weed Society of America Terminology Committee to consider the need for reviewing the WSA policy regarding the use of trademarks in scientific publications issued by the Society. He indicated that he felt the chemical industry should be responsible for developing nomenclature for new herbicides and that such responsibilities should not be included among the objectives of the WSA Terminology Committee. He then moved that the last sentence in the first paragraph on page 2 of the Terminology Committee Report be deleted. It reads as follows:

> "If common names are not developed for new herbicides, the WSA Terminology Committee will develop temporary abbreviated designations for use in all WSA publications while common names are being developed and approved."

The motion was seconded by L. S. Gleason. In discussion, W. C. Shaw indicated that the motion, if approved, would essentially eliminate the Terminology Committee's responsibilities in standardizing chemical nomenclature for herbicides. The motion was defeated. N. E. Hanson moved that the committee consider incorporating the words "in cooperation with industrial or other sponsors," so that the sentence would read as follows:

> "If common names are not developed for new herbicides, the WSA Terminology Committee will develop, in cooperation with industrial or other sponsors, temporary abbreviated designations for use in all WSA publications while common names are being developed and approved."

This motion passed. W. C. Shaw indicated that close cooperation with industry in developing herbicide nomenclature had been practiced in the past and that the suggestion was excellent.

F. M. ASHTON
W. W. ALLEN
R. BEHRENS
L. G. HOLM
W. C. SHAW, *Chairman*

*Printed on pages 477 to 521 of this issue.

SPECIAL COMMITTEE REPORTS

AUDITING COMMITTEE

F. W. Slife indicated that the Auditing Committee had examined the financial records of the Society and wished to certify that they were in excellent order.

R. L. GANTZ
F. W. SLIFE, *Chairman*

COMMITTEE ON CONSTITUTION AND BYLAWS

This committee has worked on revising the constitution of the Weed Society of America since before the Memphis meeting in 1958. A final suggested constitution* was submitted to the Executive Committee in October, 1959. This was approved by them and sent to the membership to be voted on in connection with the election of officers. It was adopted.

G. C. KLINGMAN
M. W. PARKER
C. I. SEELY
R. H. BEATTY
C. J. WILLARD, *Chairman*

*Printed on pages 482 to 486 in this issue of WEEDS.

COMMITTEE ON LOCAL ARRANGEMENTS

R. A. Fosse expressed his appreciation for the excellent assistance and cooperation which he received from all members of his committee and the management of the Cosmopolitan Hotel in making arrangements for the 1960 WSA meeting.

P. A. FRANK
J. L. FULTS
B. J. THORNTON
T. R. BARTLEY
R. A. FOSSE, *Chairman*

MEETING POLICY AND SITE COMMITTEE

At the January, 1958, meeting of the Executive Committee of the Weed Society of America, a Meeting Policy and Site Committee was appointed by President A. S. Crafts to develop guidelines for scheduling future WSA meetings and sites. After full consideration of several alternatives for annual vs biennial meetings, sites, and dates for the meetings of the Society our Committee made the following recommendations to the WSA Executive Committee:

1. That the WSA schedule biennial meetings independent of any member conference. In the future, annual meetings of the Society may be desirable after the membership is larger and the Society stronger.

2. That the WSA meet in a central area such as Kansas City, St. Louis, and Chicago, on a rotational basis and each fourth meeting in a peripheral area in the West (Denver–Salt Lake City), South (Memphis–New Orleans–Atlanta), and the East (New York–Washington–Philadelphia).

3. That the regular meetings of the Society be scheduled in February.

K. P. BUCHHOLTZ
A. S. CRAFTS
E. G. RODGERS
L. G. UTTER
C. J. WILLARD
W. B. ENNIS, JR., *Chairman*

MEMBERSHIP COMMITTEE

The expanded membership committee began to work in the latter part of 1959. In some states there was a marked response but in others the activity has not yet begun to yield results. The membership list printed in October 1959 issue of WEEDS has 100 foreign members and 526 domestic members. At the time of this meeting the 1960 membership list totals 626 of which 95 are foreign and 531 are domestic. There are 82 members who have failed to renew for 1960 at this time.

It is recommended that the expanded membership committee be reappointed as it stands to allow those members who are just getting under way to complete their tasks. If every member of the Society will make it his business to sell the Society to his associates the list of members can be increased rapidly. It is of interest to note that of the 147 authors listed in the program, 65 are not members of the Society and 5 have not renewed their membership for 1960. If people who give papers on weeds are not interested in being members of the Society then the program of the Society ought to be changed so that they are interested. Perhaps it is time to set up the requirement of membership before papers are accepted for presentation at the meeting or for publication in WEEDS.

WALTER C. JACOB, *Chairman*

SUSTAINING MEMBERSHIP COMMITTEE

During the year of 1959 there were 32 sustaining members who contributed a total of $3,200 for the support of the Weed Society of America. This is a good showing considering the fact that it was not a meeting of the Society.

For the 1960 membership campaign, 200 firms were contacted and those that did not reply to the first letter were again contacted by mail. As a result of the current campaign, $3,000 has been received from 30 firms.

It is suggested that the individual members of the conference encourage their firms to contribute to the Weed Society of America in order that it can remain in a solvent condition and continue to provide services for its members and promote the growth of the

Weed Society of America. It is also recommended that the new President write a personal letter of thanks to each of the sustaining members, thanking them for their contribution to the Sustaining Membership Committee of the Weed Society of America.

The Chairman would also like to express his thanks to the other members of the committee who have assisted him during the past two years and to especially thank Dr. Walter Jacob for the cooperation he has given to the Sustaining Membership Committee.

E. R. MARSHALL, *Chairman*

RESOLUTIONS AND NECROLOGY COMMITTEE

In the absence of P. F. Dresher, Chairman of the Resolutions and Necrology Committee, L. C. Erickson presented the report. Resolutions 1, 2, and 3 below were read by the Chairman and approved as read. After the first draft of Resolution 4 was read, L. A. Derscheid suggested that parts of this resolution were objectionable and that it needed revision. W. B. Ennis, Jr. suggested that it be returned to the committee for further study and revision and that the revised resolution be considered at the Banquet meeting of the Society. Resolution 4 was revised as printed below and approved by the Society at the Banquet meeting on February 24, 1960.

L. C. ERICKSON
D. W. LAMBERT
W. G. WESTMORELAND
P. F. DRESHER, *Chairman*

1. WHEREAS the passing of Dr. Thomas K. Pavlychenko has deprived the entire weed control program of a competent and valued worker, and

WHEREAS we individually, and as a Society will miss him and his support of our work, therefore, be it

RESOLVED, that we express our deep regret at his passing and extend to his family and close business associates our deepest sympathy; and be it further resolved that this resolution be placed in the minutes of this organization and the Secretary be instructed to transmit to his family and to Amchem Products Inc., copies of this resolution.

2. WHEREAS 1. The advancement in weed control through the use of better seed cleaning methods, improved cultural practices and better herbicides makes the production of weed free crops possible; and

2. The presence of weed seed in seed used for sowing purposes is a continuing source of dissemination; and

3. The presence of objectionable weed seeds in grain reduces its value and is an obstacle in the marketing of such grain; and

4. The screenings from grain containing weed seeds is a source of continued dissemination of such weeds; and

5. The presence of weeds in hay and forage crops reduces their value and is a continuing source of dissemination of such weeds; and

6. Lack of uniformity in the restrictions and requirements under federal and state law reveals a lack of agreement on the problem of weed control and the most practical efforts to reduce it which fact impairs the desirable progress which would seem possible with a coordinated approach: Therefore be it

RESOLVED, that there must be closer coordination of the many regulatory efforts to reduce the injury and waste caused by weeds and that this may be done through:

1. More uniformity in the noxious weed seed restrictions in the several state seed laws at least on a regional basis consistent with soil, climate and agronomic practices, coupled with more educational efforts to encourage greater use of modern seed cleaning equipment which permits the sowing of weed free seed;

2. Adoption of a suggested uniform state weed law which is practical of enforcement and gives encouragement to farmers to grow weed free crops;

3. Adoption of a uniform state feed control law which restricts the dissemination of screenings and other feed materials containing weed seeds;

4. Restrictions against the importation of screenings containing weed seeds; and

5. Coordination of the Federal and State seed, feed, and weed laws and regulations; and be it further

RESOLVED, that the Weed Society of America serve as a coordinator, urging each agency, association, and organization involved to comply with the sense of this resolution and report at the next meeting of the Society on the progress made.

3. WHEREAS any intensive effort by crop and livestock producers to suppress noxious weeds is largely nullified in many areas by reinfestation from federally owned and controlled lands; and

WHEREAS the Federal Government exercises jurisdiction over many areas contiguous to farm and grazing areas, sometimes exceeding one-third of the land area in midwest and western states, and consequently should properly recognize its responsibility as a land owner to cooperate with the organized efforts of state, county and local weed control programs; and

WHEREAS the member conferences have previously recognized the seriousness of this problem and adopted resolutions asking Congress to enact legislation to correct this situation; and

WHEREAS the 40th Annual Convention of the National Association of State Departments of Agriculture meeting at Madison, Wisconsin, September 29 to October 3, 1958, resolved that Congress

should undertake by legislation to require that every department, agency and independent establishment in the Executive Branch of the Federal Government having control of, or jurisdiction over, land located in any state, shall comply with all laws and regulations of each of the several states providing for the control of noxious weeds in the same manner that other landowners are required; therefore be it

RESOLVED, that we assembled here at the meeting of the Weed Society of America at Denver, Colorado, February 23, 1960, reaffirm our stand on said legislation and urge enactment by the 89th Congress of the needed legislation to accomplish the above stated objectives.

4. WHEREAS the Weed Society of America, a Society composed of scientists who are residents of the fifty States and who are dedicated to the advancement and promotion of the highest ethics in research and education and all other branches of the discipline of weed control, who have since the creation of the Society fostered the judicious use of all practices of weed control including the greatest caution in the application of chemical herbicides, to the end that this country now enjoys one of the most healthful, nutritious and safe food supplies of any nation on earth; and

WHEREAS scientific research must continue in its progressive efforts to not only maintain but to advance progress in providing a safe healthful and abundant food supply; and

WHEREAS copious research findings by the aggregate thousands of years of research effort by this body of scientists is now jeopardized and may become null and void by impractical and unrealistic interpretation of recently enacted legislation under the Food, Drug and Cosmetic Act, and further that such continued administrative actions constitute a serious threat to the food supply of this nation; now therefore be it

RESOLVED, that the Weed Society of America assembled at Denver, Colorado, this 24th day of February, 1960 requests immediate reconsideration by the Secretary of Health, Education and Welfare, of administrative decisions which prohibit the application of Agricultural practices and specifically weed control practices which have already been proven safe in the promotion of human health and indispensable in economical agricultural production; and be it further and respectfully

RESOLVED, that legislative acts which hinder progress be reviewed by our National Congress to the end that such legislation promotes advancement and safeguards the benefits of the past; and be it further

RESOLVED, that the Secretary of this Society be instructed to forward a copy of this resolution to the President's Scientific Advisor, the Secretary of Health, Education and Welfare, the Secretary of Agriculture and to each member of the Congress of the United States.

Constitution of the Weed Society of America

Article I—Name

This organization shall be known as the Weed Society of America.

Article II—Objectives

The objectives of this Society shall be to:

1. Encourage and promote the development of knowledge concerning weeds and their control.
2. Publish the results of meritorious research and other information of value pertaining to weeds and their control.
3. Promote unity in education, legislation, regulation, terminology, and other matters pertaining to weeds.
4. Foster high standards in weed control education, and encourage its acceptance as a major field of training.
5. Promote high ethics and good fellowship among its members.
6. Aid in the coordination of activities and cooperate with member weed control conferences, and cooperate with other societies and organizations with similar and related interests.
7. Remain a scientific organization, without the object of financial gain.

Article III—Membership

Section 1.

Membership in the Society shall be open to individuals and organizations of all nations interested in the objectives of the Society. There shall be four types of membership: (1) Active, (2) Honorary, (3) Sustaining, and (4) Conference.

Section 2.

Active members are individuals who are interested in weeds or their control and who have remitted their annual dues to the Treasurer. Active members may attend all Society meetings, vote on all matters pertaining to the Society, and hold office.

Section 3.

Honorary members are individuals who have contributed meritorious service to the study of the control of weeds and who are elected by two-thirds of the entire membership of the Executive Committee. Honorary members shall have the privileges of Active members and may receive a publication of the Society for life. Not to exceed one fourth of one percent of the Active membership may be elected to Honorary membership at any one meeting of the Society.

Section 4.

Sustaining members shall consist of persons or organizations who are interested in the objectives of the Society, who wish to participate

in the work of the Society, and who have remitted their annual dues. The names of such members shall be printed in all issues of the official publication of the Society and in the programs of the regular meetings. Each Sustaining member shall receive the Journal WEEDS.

Section 5.

Conference members are the Western Weed Control Conference, the North Central Weed Control Conference, the Northeastern Weed Control Conference, and the Southern Weed Conference as Charter Conference members. Any other similar regional organization may be admitted upon application to and approval by the Executive Committee and subsequent approval by two thirds of the members voting on a ballot sent to all members at the time of the election of officers.

Section 6.

The Executive Committee shall determine the dues for Active and Sustaining members. Honorary members shall not be asked to pay dues.

ARTICLE IV—Meetings

Meetings of the Society shall be held at the time and place designated by the Executive Committee.

ARTICLE V—Officers

Section 1.

The Executive Committee shall consist of the President, President-elect, Past President, Secretary, four members elected at large by the Society membership, and one representative from each of the member Conferences. The Editor of "WEEDS" and the Treasurer-Business Manager of the Society shall also be ex officio members of the Executive Committee.

Section 2.

The elected officers of the Society shall be a President, a President-elect, who serves as Vice-President, a Secretary, and four members of the Executive Committee. The President-elect shall succeed to the office of President immediately after serving a term as Vice-President. The duties of these officers shall be those usually performed by such officers.

Section 3.

The President, President-elect, and Secretary shall begin their duties at the close of the regular business meeting at which they are installed, and shall remain in office until the close of the next regular business meeting of the Society. The President shall not be eligible for a second term. The elected members of the Executive Committee shall begin their duties at the close of the meeting at which they are installed.

Section 4.

The Executive Committee shall elect a Treasurer–Business Manager and an Editor, to serve at the pleasure of that committee.

ARTICLE VI—Election of Officers

Section 1.

The Nominating Committee shall consist of one member from each member Conference plus a chairman. The Executive Committee shall select the members of the Nominating Committee and the chairman. The Nominating Committee shall present at least two names for each office to be filled.

At the first election after this section takes effect, the Nominating Committee shall present at least six names for members of the Executive Committee. The two receiving the highest number of votes shall be elected for four-year terms; the two receiving the next highest numbers of votes shall be elected for two-year terms. Thereafter members of the Executive Committee not members ex officio shall serve for four-year terms.

When this section first takes effect, two Conferences, to be selected by lot, shall select Executive Committee members to hold office for two years, and two shall select members to serve for four-year terms. Thereafter, all Conference representatives shall be selected for four-year terms.

Section 2.

No member's name shall be placed on the ballot by the Nominating Committee without his written consent.

Section 3.

At least four weeks prior to each regular meeting of the Society, ballots shall be mailed to all Active and Honorary members. All ballots received one week before the first day of the meeting shall be counted by the Nominating Committee. The chairman and at least one other member of the Nominating Committee shall do the counting. If these persons are not able to attend the meeting, proxies shall be appointed by the President.

Section 4.

If an officer other than the President or a representative of a Conference on the Executive Committee cannot serve the full elected term, the vacancy shall be filled for the interim by appointment by the Executive Committee. The President-elect shall serve as President if the President becomes unable to serve. This service shall not constitute his term as President. The replacement of a conference representative shall be the responsibility of the Conference concerned.

ARTICLE VII—Standing Committees

Section 1.

There shall be seven standing committees: Program, Finance, Terminology, Editorial, Education, Legislative, and Public Relations. The President or the Executive Committee may appoint other committees to carry out Society business. Committee members must be active members of the Society. The President shall be an ex officio member of all committees.

Section 2.

The Program Committee shall consist of the President-elect as Chairman and four additional members to be appointed by the President.

Section 3.

The Finance Committee shall consist of a Chairman and four members appointed by the President, one each year for a term of five years.

Section 4.

The Terminology Committee shall consist of a Chairman and four members appointed by the President, one each year for a term of five years.

Section 5.

The Editorial Committee shall consist of the Editor and four or more members appointed by the Editor.

Section 6.

The Legislative Committee shall consist of a Chairman and four members appointed by the President, one each year for a term of five years.

Section 7.

The Education Committee shall consist of a Chairman and four members appointed by the President, one each year for a term of five years.

Section 8.

The Public Relations Committee shall consist of a Chairman and four members appointed by the President, to serve until a new President takes office.

ARTICLE VIII—Endowment Funds

Endowment funds for specific purposes may be established at any time by the Executive Committee subject to the provisions of the Constitution and the By-Laws of the Society. It is understood that the principal of the endowment funds shall be kept productively invested, only the incomes therefrom being available.

Bequests and gifts of funds or securities accepted without obligation by the Executive Committee shall be administered by the Executive Committee with due regard to the wishes of the donor.

ARTICLE IX—Auditing of Accounts

Accounts of the Treasurer–Business Manager and others entrusted with funds shall be audited annually by a qualified auditor or auditing committee.

The Auditor's reports shall be submitted to the Society at the general business meeting.

ARTICLE X—Publications

Section 1.

One or more publications may be published by the Society as authorized by the Executive Committee. Each publication shall be under the direction of an Editor who shall be appointed by the Executive Committee.

Section 2.

The official Journal of the Society shall be called WEEDS and shall carry all official notices, committee reports, and other items of Society business. The policies of the Journal shall be established jointly by the Executive Committee and the Editorial Committee. Any member of the Society may contribute to the Journal. Acceptance of such contributions rests with the Editorial Committee.

ARTICLE XI—Amendments

Section 1.

Amendments to this constitution may be proposed by any ten members of the Society. The Executive Committee shall consider such proposed amendments and submit them to the members with their recommendations, either in a special mail ballot, or at the next regular election. A majority of the Executive Committee may also initiate amendments, to be voted on in the same way.

Section 2.

Adoption of a proposed amendment shall require a two-thirds vote of those members voting on the amendment.

Report of the Terminology Committee Weed Society of America

One of the most acute problems in weed control terminology is the need for the development and adoption of standardized common names for herbicides and weeds. Similar terminology problems occur in every scientific field which involves the use of agricultural chemicals on plants and animals. The lack of standardized terminology inhibits effective communication in science and delays the acceptance of results of scientific research.

The objectives of the Terminology Committee of WSA are (1) to coordinate the terminology in the field of weed control, including the selection and approval of common names for herbicides and weeds, and (2) to review, revise, and develop standardized terms, abbrevations, and definitions used in the field of weed control.

The Committee recognizes that during the discovery, development, and use of herbicides at least two nomenclature phases occur in the selection of a designation to be used for a herbicidal chemical. The first phase involves the synthesis of new chemicals and the discovery of their biological properties. When new chemicals are first synthesized they are given chemical names. However, when new chemicals are evaluated for their biological properties they are usually given code numbers to facilitate data processing and to conceal their identity until patent protection has been secured. The second phase involves releasing their chemical names and herbicidal properties to research personnel other than those of the organization responsible for their discovery. The Weed Society of America strongly urges the chemical industry to develop common names for new herbicides as early as possible after their discovery. If common names for new herbicides are developed early in research and development programs of the organizations introducing new herbicides, the use of code numbers and trademark names in the scientific literature can be avoided and the development of WSA abbreviated number-letter designations will not be necessary. When common names are not developed by organizations introducing new herbicides, a variety of abbreviations and designations occur, resulting in confusion. If common names are not developed for new herbicides, the WSA Terminology Committee will develop, in cooperation with industrial and other sponsors, temporary abbreviated designations for use in all WSA publications until common names are developed and approved.

A Committee known as the American Standards Association Sectional Committee on Common Names for Pest Control Chemicals K62 (ASA–K62) has been organized for the purpose of adopting and standardizing common names for chemicals used as herbicides, insecticides, fungicides, rodenticides, nematocides, defoliants, and plant regulators. The American Standards Association (ASA) is also a member of the International Standards Organization (ISO) and the ASA–K62 Committee will work with foreign countries through ISO in its efforts to standardize common names for agricultural chemicals throughout the world.

The Weed Society of America is a member of ASA–K62, which is composed of representatives from scientific societies and all other organizations interested in agricultural chemical nomenclature. The Terminology Committee of WSA is represented on the ASA–K62 Committee by the WSA Terminology Committee Chairman and two alternates. We have participated in meetings of ASA–K62 relating to herbicide terminology and aided in the development of the procedure for developing common names for pest control chemicals which was published in WEEDS.

Sponsors of common names for herbicidal chemicals should prepare a "Statement" in accordance with the procedures established by ASA–K62 and published in WEEDS 4:278–284 (1956) and forward ten copies of the Statement to the Chairman of the WSA Terminology Committee for action. If the common name proposed by the sponsor is accepted by the Terminology Committee of WSA, the Committee will recommend that the sponsor prepare copies of the Statement for submittal to the American Standards Association Sectional Committee on Common Names for Pest Control Chemicals, K62 for formal consideration for approval. Copies of the "American Standard Procedure for the Acceptance of an American Standard Common Name for a Pest Control Chemical" for use by sponsors preparing Statements on common names of herbicides for ASA approval may be obtained by writing to the Secretary, ASA Sectional Committee K62, American Standards Association, Incorporated, 70 East Forty-fifth Street, New York 17, N. Y.

This introduction and the detailed reports (A to E) which follow were prepared by the Terminology Committee and Sub-committees, Weed Society of America, January 11, 1960.

Comments and suggestions on (1) Herbicide Nomenclature should be sent to W. C. Shaw, Chairman of the WSA Terminology Committee; (2) Abbreviations, Terms, and Definitions should be sent to R. Behrens, Chairman of the Sub-committee on Standardization of Abbreviations, Terms, and Definitions, and (3) Common and Botanical Names of Weeds should be sent to R. A. Darrow, Chairman of the Sub-committee on Standardization of Common and Botanical Names of Weeds.

WSA Terminology Committee

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A. Suggested terminology guides for authors to follow in preparing manuscripts for publication in all publications of WSA.

The editorial board will not approve manuscripts for publication in any publication of WSA in which trade names, code names, code numbers, or abbreviated names are used without identifying the active ingredient in the herbicide. The first time a herbicide is mentioned in a manuscript, research report, abstract, or other publication, the full chemical name of the herbicide must be given, followed by the WSA accepted common name or designation. Herbicides for which WSA accepted common names or designations have not been approved should be designated by their full chemical names. The author may, however, assign a code to the chemical for subsequent use in his manuscript.

The exact derivative of compounds should be used. For example, in the body of manuscripts use diethanolamine salt of 2,4–D; isopropyl ester of 2,4–D; butoxyethanol ester of 2,4–D; while for tabular presentation use 2,4–D, sodium salt; 2,4–D, butyl ester; etc. Avoid the use of sodium 2,4–D; 2,4–D sodium; amine 2,4–D; 2,4–D amine; LV ester of 2,4–D; 2,4–D ester, and others.

Rates of application should always be stated in terms of the acid equivalent when applicable and, when not applicable, in terms of the active ingredient.

Designations for chemical compounds involving a combination of numbers and letters should be written with capital letters without spacing or periods. The numbers should be separated by commas, except where brackets or parenthesis occur, and the numbers and letters should be separated by a dash (–). For example: 2,4–D, MCPA, 2,3,6–TBA, 4–(2,4–DB), 4–(4–CPB). In writing accepted common names, lower case letters should be used. For example: monuron, diuron, dalapon.

In acknowledging the source of experimental compounds it is suggested this be done in a footnote rather than by the use of trade names in the body of manuscripts. For example: "In these studies three compounds, 2,4–D,[1] MCPA,[1] and 2,4,5–T,[1] were applied at three rates and at three stages of growth.

[1]The authors wish to acknowledge the cooperation of the ABC Chemical Company, Hometown, Pa. in supplying the compounds for these investigations."

B. Nomenclature for chemicals used as herbicides.

Chemical name	Designations accepted for use in all publications of WSA
2,4–dichlorophenoxyacetic acid	2,4–D
2–methyl–4–chlorophenoxyacetic acid	MCPA
2,4,5–trichlorophenoxyacetic acid	2,4,5–T
3,4–dichlorophenoxyacetic acid	3,4–DA
4–chlorophenoxyacetic acid	4–CPA
2–(2,4–dichlorophenoxy)propionic acid	2–(2,4–DP)
2–(2–methyl–4–chlorophenoxy)propionic acid	2–(MCPP)
2–(2,4,5–trichlorophenoxy)propionic acid	silvex (sĭl′ věks)
2–(3,4–dichlorophenoxy)propionic acid	2–(3,4–DP)
2–(4–chlorophenoxy)propionic acid	2–(4–CPP)
4–(2,4–dichlorophenoxy)butyric acid	4–(2,4–DB)
4–(2–methyl–4–chlorophenoxy)butyric acid	4–(MCPB)
4–(2,4,5–trichlorophenoxy)butyric acid	4–(2,4,5–TB)
4–(3,4–dichlorophenoxy)butyric acid	4–(3,4–DB)
4–(4–chlorophenoxy)butyric acid	4–(4–CPB)
sodium 2,4–dichlorophenoxyethyl sulfate	sesone[a] (sĕs′ ōn)
sodium 2,4,5–trichlorophenoxyethyl sulfate	2,4,5–TES
sodium 2–methyl–4–chlorophenoxyethyl sulfate	MCPES
2,4–dichlorophenoxyethyl benzoate	2,4–DEB
trichloroacetic acid	TCA
2,2–dichloropropionic acid	dalapon (dăl′ ăpŏn)
2,2,3–trichloropropionic acid	2,2,3–TPA
dichloral urea	DCU
2–chloro–*N*,*N*–diallylacetamide	CDAA
2–chloro–*N*,*N*–diethylacetamide	CDEA
isopropyl *N*–phenylcarbamate	IPC
isopropyl *N*–(3–chlorophenyl)carbamate	CIPC
sec–butyl *N*–(3–chlorophenyl)carbamate	BCPC
4–chloro–2–butynyl *N*–(3–chlorophenyl)carbamate	barban[a] (băr′ băn)
2–chloroallyl diethyldithiocarbamate	CDEC
3–phenyl–1,1–dimethylurea	fenuron (fĕn′ ū rŏn)
3–phenyl–1,1–dimethylurea trichloroacetate	fenuronTCA
3–(*p*–chlorophenyl)–1,1–dimethylurea	monuron (mŏn′ ū rŏn)
3–(*p*–chlorophenyl)–1,1–dimethylurea trichloroacetate	monuronTCA
3–(3,4–dichlorophenyl)–1,1–dimethylurea	diuron (dī′ ū rŏn)
1–*n*–butyl–3–(3,4–dichlorophenyl)–1–methylurea	neburon (nĕb′ ū rŏn)
pentachlorophenol	PCP
4,6–dinitro–*o*–*sec*–butylphenol	DNBP
4,6–dinitro–*o*–*sec*–amylphenol	DNAP
3,5–dinitro–*o*–cresol	DNC
2,3,6–trichlorobenzoic acid	2,3,6–TBA[b]
2,3,5,6–tetrachlorobenzoic acid	2,3,5,6–TBA[b]
N–1–naphthylphthalamic acid	NPA
3,6–endoxohexahydrophthalic acid	endothal[a] (ĕn′ dō thăl)
phenylmercuric acetate	PMA
potassium cyanate	KOCN
hexachloroacetone	HCA
isopropylxanthic acid	IPX
1,2–dihydropyridazine–3,6–dione (maleic hydrazide)	MH
trichlorobenzene	TCB
o–dichlorobenzene	DCB
3–amino–1,2,4–triazole	amitrole[a] (ăm′ ĭ trōl)
3–amino–1,2,4–triazole—ammonium thiocyanate	amitrole–T[c]
octachlorocyclohexenone	OCH

Chemical name	Designations accepted for use in all publications of WSA
ethyl xanthogen disulfide	EXD
monomethylarsonic acid	MAA
2–(2,4,5–trichlorophenoxy)ethyl 2,2–dichloro–propionate	erbon (ûr′ bŏn)
polychlorobenzoic acid	PBA[b]
2–chloroethyl *N*–(3–chlorophenyl)carbamate	CEPC
1–chloro–2–propyl *N*–(3–chlorophenyl)carbamate	CPPC
ethyl *N*,*N*–di–*n*–propylthiolcarbamate	EPTC
ethyl bis(2–ethylhexyl)phosphinate	EBEP
P,*P*–dibutyl–*N*,*N*–diisopropylphosphinic amide	DIPA
disodium monomethylarsonate	DMA
chlorate–borate–monuron mixtures	CBMM[c]
chlorate–borate–fenuron mixtures	CBFM[c]
chlorate–borate–diuron mixtures	CBDM
chlorate–borate mixtures	CBM[c]
borate–monuron mixtures	BMM[c]
borate–2,4–D mixtures	BDM[c]
ammonium sulfamate	AMS
2,3–dichloro–1,4–naphthoquinone	dichlone (dī′ klōn)
sodium—methyldithiocarbamate	SMDC
3,5–dimethyltetrahydro–1,3–5,2*H*–thiadiazine–2–thione	DMTT
3–amino–2,5–dichlorobenzoic acid	amiben[a] (ăm ĭ′ bĕn′)
2,3,6–trichlorophenylacetic acid	fenac
2–chloro–4,6–bis(ethylamino)–*s*–triazine	simazine[a] (sĭm′ ă zēn)
2–chloro–4–ethylamino–6–isopropylamino–*s*–triazine	atrazine[a] (ă′ tră zēn)
2–chloro–4–diethylamino–6–isopropylamino–*s*–triazine	ipazine[a] (ĭ′ pă zēn)
2–chloro–4–diethylamino–6–ethylamino–*s*–triazine	trietazine[a] (trī′ ĕ tă zēn)
2–chloro–4,6–bis(isopropylamino)–*s*–triazine	propazine[a] (prō′ pă zēn)
2–chloro–4,6–bis(diethylamino)–*s*–triazine	chlorazine[a] (klō′ ră zĕn)
2–methoxy–4,6–bis(ethylamino)–*s*–triazine	simetone[a] (sĭ′ mĕ tōn)
2–methoxy–4,6–bis(isopropylamino)–*s*–triazine	prometone[a] (prō′ mĕ tōn
2–methoxy–4–ethylamino–6–isopropylamino–*s*–triazine	atratone[a] (ă′ tră tōn)
1,1′–ethylene–2,2′–dipyridylium dibromide	diquat[a] (dī′ kwăt)
acrylaldehyde	acrolein (á krō′ lē–ĭn)
tris(2,4–dichlorophenoxyethyl) phosphite	2,4–DEP

[a]Common names "tentatively accepted" by the Terminology Committee of WSA, pending formal approval by ASA.

[b]These herbicides are usually available for use as mixed isomers. When possible the isomers should be identified, the amount of each isomer in the mixture specified, and the source of the experimental chemicals given.

[c]These abbreviations are used to designate mixtures used as soil sterilants. The writer should indicate in a footnote the percentage composition of the product. For example: sodium chlorate—40.0%, sodium metaborate—57.0%, and monuron 1.0%.

C. Abbreviations of terms used in weed control.

The use of the following abbreviations is recommended and unless otherwise indicated, should be lower case letters without spacing or periods.

Terms	Abbreviations
acre or acres	A
acid equivalent	ae
acid equivalent per 100 gallons	aehg
active ingredient	ai
active ingredient per 100 gallons	aihg
bushel or bushels	bu
cubic	cu
cubic feet per second	cfs
diameter	diam
feet per minute	fpm
foot or feet	ft
gallon or gallons	gal
gallons per acre	gpa
gallons per hour	gph
gallons per minute	gpm
gram or grams	gm
hour or hours	hr
height	ht
inch or inches	in
liter or liters	L
melting point	mp
mile or miles	mi
miles per hour	mph
milligram or milligrams	mg
milliliter or milliliters	ml
millimeter or millimeters	mm
minute or minutes	min
ounce or ounces	oz
parts per million by volume	ppmv
parts per million by weight	ppmw
pint or pints	pt
pound or pounds	lb
pounds per acre	lb/A
pounds per square inch	psi
precipitate	ppt
quart or quarts	qt
revolutions per minute	rpm
rod or rods	rd
specific gravity	sp gr
square	sq
technical	tech
temperature	temp
ton or tons	T
weight	wt
Weed Society of America	WSA
North Central Weed Control Conference	NCWCC
Northeastern Weed Control Conference	NEWCC
Southern Weed Conference	SWC
Western Weed Control Conference	WWCC

D. Definitions of terms used in weed control.

Term	Definition

Annual. A plant that completes its life cycle from seed in 1 year.

Band application. An application to a continuous restricted area such as in or along a crop row rather than over the entire field area.

Basal treatment. A treatment applied to the stems or trunks of plants at and just above the ground line.

Bed. (1) A narrow flat-topped ridge on which crops are grown with a furrow on each side for drainage of excess water. (2) An area in which seedlings or sprouts are grown before transplanting.

Bed-up. To build up beds or ridges with a tillage implement.

Biennial. A plant that completes its life cycle in 2 years. The first year its produces leaves and stores food. The second year it produces fruits and seeds.

Broadcast application. An application over an entire area.

Blind cultivation. Cultivation before a seeded or planted crop emerges.

Brush control. Control of woody plants.

Carrier. The liquid or solid material added to a chemical compound to facilitate its application in the field.

Compatible. Compounds are said to be compatible when they can be mixed and applied satisfactorily with effective results.

Concentration. Refers to the amount of active ingredient or acid equivalent in a given volume or weight of diluent. Recommendations and specifications for concentration of herbicides should be on the basis of pounds per unit volume of liquid diluent or percent by weight of solid diluents.

Contact herbicide. A herbicide that kills primarily by contact with plant tissue rather than as a result of translocation.

Defoliator or Defoliant. A compound which causes the leaves, or foliage, to drop from the plant.

Desiccant. A compound that promotes dehydration or removal of moisture from plant tissue.

Diluent. Any liquid or solid material serving to dilute an active ingredient in the preparation of a formulation.

Directed application. An application to a restricted area such as a row or bed, or at the base of plants.

Dormant. State of inhibited growth of seeds or other plant organs due to internal causes.

Emulsifying agent. A surface active material which facilitates the suspension of one liquid in another.

Emulsion. The suspension of one liquid as minute globules in another liquid; for example, oil dispersed in water.

Term	Definition

Epinasty. Increased growth on upper surface of a plant organ or part (especially of leaves) causing it to bend downward.

Growth stages of cereal crops. (1) Tillering—when additional shoots are developing from the lower buds. (2) Jointing—when stem internodes begin elongating rapidly. (3) Boot—when upper leaf sheath swells due to the growth of developing spike or panicle. (4) Heading—when seed head is emerging from the upper leaf sheath.

Growth regulator. An organic substance effective in minute amounts for modifying plant processes.

Herbaceous plant. A vascular plant that does not develop woody tissue.

Herbicide. A phytotoxic chemical used for killing or inhibiting the growth of plants.

Miscible liquids. Two or more liquids capable of being mixed, which will remain mixed under normal conditions.

Perennial. A plant that lives for more than 2 years.

Non-selective herbicide. A chemical that is generally toxic to plants without regard to species.

Noxious weed. A weed arbitrarily defined by law as being especially undesirable, troublesome, and difficult to control. Definition of the term "noxious weed" will vary according to legal interpretations.

Phytotoxic. Poisonous to plants.

Post-emergence. After emergence of specified weed or crop.

Pre-emergence. Prior to emergence of specified weed or crop.

Pre-planting. Any time before the crop is planted.

Rate. Rate refers to the amount of active ingredient or acid equivalent of a herbicide applied per unit area (such as one acre). Rate is preferred to the occasionally used terms, dosage and application.

Selective herbicide. A chemical that is more toxic to some plant species than to others.

Soil application. Application of chemical made primarily to the soil surface rather than to vegetation.

Soil incorporation. Mechanical mixing of the herbicide with the soil.

Soil injection. Mechanical placement of the herbicide beneath the soil surface with a minimum of mixing or stirring.

Soil sterilant. A herbicide that prevents the growth of plants when present in the soil. Soil sterilization may be temporary or relatively permanent.

Spray drift. The movement of airborne spray particles from the intended area of application.

Term	Definition

Surfactant. A material which facilitates, and accentuates the emulsifying, dispersing, spreading, wetting, and other surface-modifying properties of herbicide formulations.

Suspension. A system consisting of very finely divided solid particles dispersed in a solid, liquid or gas.

Synergism. Cooperative action of different chemicals such that the total effect is greater than the sum of the independent effects.

Translocated herbicide. A translocated herbicide is one that moves within the plant a considerable distance from the point of application.

Vapor drift. The movement of herbicidal vapors from the area of application.

Volatile. A compound is said to be volatile when it evaporates, or vaporizes (changes from a liquid or solid to a gas) at ordinary temperatures on exposure to the air.

Wetting agent. A compound which when added to a spray solution causes it to contact plant surfaces more thoroughly.

Weed. A plant growing where it is not desired.

Weed eradication. The complete elimination of all live plants, plant parts, and seeds of a weed infestation from an area.

Weed control. The process of limiting weed infestations so that crops can be grown profitably or other operations can be conducted efficiently.

E. Standardized names of weeds

In recognition of the need for a standardized nomenclature for weeds occurring in continental United States and Canada, the Subcommittee on Standardization of Common and Botanical Names of Weeds was appointed in May 1957, as a unit of the Terminology Committee of the WSA. The objectives of the subcommittee have been to develop standardized checklists of weeds for use in technical and popular publications by members of the WSA and associated regional weed control conferences. It is believed that the availability and use of such a standardized nomenclature will provide a basis for consistency and clarification in the general use of common and botanical names of weeds among all technical workers in agriculture and by the general public.

In the development of a standardized list of common names of weeds, the committee recognizes that different vernacular names have been applied to the same species in various regions and that the same common name has been used for more than one species or generic group. Accepted common names have been selected insofar as possible with preference to names already in general use over the major area of occurrence or importance. In large generic groups, a basic common name has been selected except in groups with two or more well established root names. Hyphenated and possessive names have largely been eliminated. Preference is given to unified or linked names such as eveningprimrose, morningglory, pepperweed, and canarygrass for groups of species with a basic common name.

This initial report of the Subcommittee includes the approved common and botanical names of the more important annual and perennial herbaceous weeds of the United States and Canada, including grasses and grasslike plants. Woody plants, vines, aquatic weeds and other groups will be treated in subsequent reports.

This list includes all noxious plants recognized by the various states according to the October 1957 publication of the Agricultural Marketing Service, "State Noxious-Weed Seed Requirements recognized in the Administration of the Federal Seed Act" and subsequent revisions in October, 1958 and November 1959. Acknowledgment is given to Dr. O. L. Justice and Dr. E. W. Sundermeyer of the Grain Division of the Agricultural Marketing Service for their assistance in reviewing preliminary drafts of this report, and to the late Dr. S. F. Blake of the Crops Research Division, Agricultural Research Service, for assistance in checking the scientific nomenclature. Recognition is also given to the lists of plants prepared by the North Central Regional Technical Committee (Univ. Illinois Agric. Expt. Sta. Circ. 718), the Western Weed Conference list prepared by the late Dr. A. N. Steward of Oregon State College and the authorized list of Common Names of Weeds prepared for the Western Canadian Weed Control Conference by A. C. Budd, Dominion Experiment Station, Swift Current, Saskatchewan in May, 1950.

Plant names are listed alphabetically according to (1) common

name, with corresponding scientific name including the authority, and (2) genus and species, with corresponding common name. In the list of common names, cross reference has been made to alternative names in generic groups. For example, after the entry amaranth, the notation, *see also* pigweed, will permit the reader to find other listed species of the same group. Some names in general usage but not accepted in the present standardized list are also included with reference to the appropriate accepted common name. For example, under the entry bitterweed, reference is made to bitter rubberweed and bitter sneezeweed, accepted names for two species commonly called bitterweed in various regions.

Scientific nomenclature is based on Gray's Manual of Botany, 8th edition, revised by M. L. Fernald, 1950; Manual of the Grasses of the United States, by A. S. Hitchcock and Agnes Chase, 1950; and other recent taxonomic studies and manuals. Synonyms are included in the alphabetized list of genera and species in a few instances in which the replaced name has been in general use.

ANNUAL AND PERENNIAL HERBACEOUS WEEDS

(arranged alphabetically by common name)

Common Name	Scientific Name
agrimony	*Agrimonia gyrposepala* Wallr.
alyssum, hoary	*Berteroa incana* (L.) DC.
amaranth	
green (*see* smooth pigweed)	
Palmer	*Amaranthus palmeri* S. Wats.
spiny	*Amaranthus spinosus* L.
see also pigweed	
arrowgrass	
marsh	*Triglochin palustris* L.
seaside	*Triglochin maritima* L.
artichoke, Jerusalem	*Helianthus tuberosus* L.
aster	
manyflowered	*Aster ericoides* L.
slender	*Aster exilis* Ell.
spiny	*Aster spinosus* Benth.
white field	*Aster simplex* Willd.
white heath	*Aster pilosus* Willd.
woody	*Aster parryi* Gray
atamosco lily	*Zephyranthes atamasco* (L.) Herb.
Austrian fieldcress	*Rorippa austriaca* (Crantz) Bess.
Austrian peaweed	*Swainsona salsula* (Pall.) Taub.
baneberry, red	*Actaea rubra* (Ait.) Willd.
barley	
foxtail	*Hordeum jubatum* L.
little	*Hordeum pusillum* Nutt.
meadow	*Hordeum brachyantherum* Nevski
wild	*Hordeum leporinum* Link.
barnyard grass	*Echinochloa crusgalli* (L.) Beauv.
bassia, fivehook	*Bassia hyssopifolia* (Pall.) Kuntze
beancaper, Syrian	*Zygophyllum fabago* L.
beardgrass, bushy	*Andropogon glomeratus* (Walt.) B.S.P.
bedstraw	*Galium aparine* L.
northern	*Galium boreale* L.

Common Name	Scientific Name
rough	*Galium asprellum* Michx.
smooth	*Galium mollugo* L.
yellow	*Galium verum* L.
beeplant, Rocky Mountain	*Cleome serrulata* Pursh
beggarticks	
devils	*Bidens frondosa* L.
tall	*Bidens vulgata* Greene
see also Spanishneedles	
beggarweed, Florida	*Desmodium tortuosum* (Sw.) DC.
bellflower, creeping	*Campanula rapunculoides* L.
Bermudagrass	*Cynodon dactylon* (L.) Pers.
bindweed	
field	*Convolvulus arvensis* L.
hedge	*Convolvulus sepium* L.
bittercress	*Cardamine* spp.
bitterweed (*see* bitter rubberweed, bitter sneezeweed)	
black medic	*Medicago lupulina* L.
blackeyed susan	*Rudbeckia serotina* Nutt.
bluegrass	
annual	*Poa annua* L.
Canada	*Poa compressa* L.
Kentucky	*Poa pratensis* L.
bluestem, little	*Andropogon scoparius* Michx.
blueweed, Texas	*Helianthus ciliaris* DC.
see also blue thistle	
boneset	*Eupatorium perfoliatum* L.
bouncingbet	*Saponaria officinalis* L.
bracken fern	*Pteridium aquilinum* (L.) Kuhn
bristlegrass	
bur	*Setaria vertilcillata* (L.) Beauv.
knotroot	*Setaria geniculata* (Lam.) Beauv.
see also foxtail	
brome	
downy	*Bromus tectorum* L.
Japanese	*Bromus japonicus* Thunb.
red	*Bromus rubens* L.
ripgut	*Bromus rigidus* Roth
upright	*Bromus racemosus* L.
see also chess, cheat	
broomsedge	*Andropogon virginicus* L.
broomweed	
common	*Gutierrezia dracunculoides* (DC.) Blake
Texas	*Gutierrezia texana* Torr. & Gray
see also snakeweed	
buckwheat	
Tartary	*Fagopyrum tataricum* (L.) Gaertn.
wild	*Polygonum convolvulus* L.
buffalobur	*Solanum rostratum* Dunal
bugleweed	*Lycopus uniflorus* Michx.
burclover, California	*Medicago hispida* Gaertn.
burcucumber	*Sicyos angulatus* L.
burdock	
common	*Arctium minus* (Hill) Bernh.
great	*Arctium lappa* L.
buttercup	
bulbous	*Ranunculus bulbosus* L.
celeryleaf	*Ranunculus scleratus* L.
corn	*Ranunculus arvensis* L.
creeping	*Ranunculus repens* L.
smallflower	*Ranunculus abortivus* L.

Common Name	Scientific Name
tall	*Ranunculus acris* L.
butterfly milkweed	*Asclepias tuberosa* L.
buttonweed (*see* poorjoe)	
camomile (*see* chamomile)	
camphorweed	*Heterotheca subaxillaris* (Lam.) Britt. & Rusby
campion	
bladder	*Silene cucubalus* Wibel
red	*Lychnis dioica* L.
see also catchfly	
canarygrass	*Phalaris canariensis* L.
reed	*Phalaris arundinacea* L.
carelessweed (*see* Palmer amaranth)	
carrot, wild	*Daucus carota* L.
catchfly	
hairy	*Silene dichotoma* Ehrh.
nightflowering	*Silene noctiflora* L.
sleepy	*Silene antirrhina* L.
see also bladder campion	
carpetweed	*Mollugo verticillata* L.
catnip	*Nepeta cataria* L.
catsear, spotted	*Hypochoeris radicata* L.
chamomile	
corn	*Anthemis arvensis* L.
false	*Matricaria maritima* L. var. *agrestis* (Knaf) Wilmott
yellow	*Anthemis tinctoria* L.
charlock (*see* wild mustard)	
cheat	*Bromus secalinus* L.
chess	
hairy	*Bromus commutatus* Schrad.
soft	*Bromus mollis* L.
see also brome, cheat	
chickweed	
common	*Stellaria media* (L.) Cyrill.
field	*Cerastium arvense* L.
mouseear	*Cerastium vulgatum* L.
chicory	*Cichorium intybus* L.
cinquefoil	
common	*Potentilla canadensis* L.
downy	*Potentilla intermedia* L.
rough	*Potentilla norvegica* L.
silvery	*Potentilla argentea* L.
sulphur	*Potentilla recta* L.
clammyweed	*Polanisia graveolens* Raf.
western	*Polanisia trachysperma* Torr. & Gray
cockle	
corn	*Agrostemma githago* L.
cow	*Saponaria vaccaria* L.
white	*Lychnis alba* Mill.
cocklebur	
beach	*Xanthium echinatum* Murr.
common	*Xanthium pensylvanicum* Wallr.
oriental	*Xanthium chinense* Mill.
spiny	*Xanthium spinosum* L.
coffeeweed	*Daubentonia texana* Pierce
coneflower	
prairie	*Ratibida columnaris* (Sims) D. Don
tall	*Rudbeckia laciniata* L.
coreopsis *see* (tickseed)	
corn spurry	*Spergula arvensis* L.

Common Name	Scientific Name
cornflower	*Centaurea cyanus* L.
couchgrass (*see* quackgrass)	
crabgrass	
large-	*Digitaria sanquinalis* (L.) Scop.
slender-	*Digitaria filiformis* (L.) Koel.
smooth-	*Digitaria ischaemum* (Schreb.) Muhl.
cress	
Austrian field-	*Rorippa austriaca* (Crantz) Bess.
bitter-	*Cardamine* spp.
hoary	*Cardaria draba* (L.) Desv.
marsh-	*Rorippa islandica* (Oeder) Borbas
swine-	*Coronopus didymus* (L.) Smith
crotalaria, showy	*Crotalaria spectabilis* Roth
croton	
Lindheimer	*Croton lindheimeri* (Engelm. & Gray) Wood
Texas	*Croton texensis* (Klotsch) Muell. Arg.
woolly	*Croton capitatus* Michx.
crownbeard	*Verbesina encelioides* Gray var. *exauriculata* Rob. & Greenm.
cucumber	
western wild	*Echinocystis oregona* (Torr. & Gray) Cogn.
wild	*Echinocystis lobata* (Michx.) Torr. & Gray
cudweed, low	*Gnaphalium uliginosum* L.
cuphea, clammy	*Cuphea petiolata* (L.) Koehne
cupplant	*Silphium perfoliatum* L.
daisy	
English	*Bellis perennis* L.
oxeye	*Chrysanthemum leucanthemum* L.
Dallisgrass	*Paspalum dilatatum* Poir.
dandelion	*Taraxacum officinale* L.
fall (*see* fall hawkbit)	
false (*see* spotted catsear)	
darnel	*Lolium temulentum* L.
dayflower	*Commelina communis* L.
deadnettle	
red	*Lamium purpureum* L.
spotted	*Lamium maculatum* L.
see also henbit	
deathcamas	
foothill	*Zigadenus paniculatus* (Nutt.) Wats.
meadow	*Zigadenus venenosus* Wrts.
dock	
broadleaf	*Rumex obtusifolius* L.
cluster	*Rumex conglomeratus* Murr.
curly	*Rumex crispus* L.
Mexican	*Rumex mexicanus* Meisn.
patience	*Rumex patientia* L.
smooth	*Rumex altissimus* Wood
veiny	*Rumex venosus* Pursh
western	*Rumex occidentalis* S. Wats.
see also sorrel, red sorrel	
dodder	
Chilean	*Cuscuta racemosa* Martius var. *chileana* Engelm.
clover	*Cuscuta epithymum* Murr.
field	*Cuscuta campestris* Yunck.
flax	*Cuscuta epilinum* Weihe
largeseed	*Cuscuta indecora* Choisy
smallseed	*Cuscuta planiflora* Tenore
swamp	*Cuscuta gronovii* Willd.

Common Name	Scientific Name
dogbane, spreading	*Apocynum androsaemifolium* L.
hemp (*see* Indian hemp)	
dropseed, annual	*Sporobolus neglectus* Nash
eryngo, Hooker	*Eryngium hookeri* Walp.
eveningprimrose	
common	*Oenothera biennis* L.
cutleaf	*Oenothera laciniata* Hill
pale	*Oenothera pallida* Lindl.
prairie	*Oenothera albicaulis* Pursh
everlasting	
fragrant	*Gnaphalium obtusifolium* L.
pearly	*Anaphalis margaritacea* (L.) Benth.
plantainleaf	*Antennaria plantaginifolia* (L.) Hook.
see also cudweed	
falseflax	
largeseed	*Camelina sativa* (L.) Crantz
smallseed	*Camelina microcarpa* Andrz.
fennel	
common	*Foeniculum vulgare* Gaertn.
dog	*Eupatorium capillifolium* (Lam.) Small
see also mayweed	
fern, bracken	*Pteridium aquilinum* (L.) Kuhn
fescue	
foxtail	*Festuca megalura* Nutt.
rattail	*Festuca myuros* L.
sixweeks	*Festuca octoflora* Walt.
fiddleneck	
coast	*Amsinckia intermedia* Fisch. & May.
Douglas	*Amsinckia douglasiana* A. DC.
western	*Amsinckia tessellata* Gray
fieldcress, Austrian	*Rorippa austriaca* (Crantz) Bess.
filaree	
broadleaf	*Erodium botrys* (Cav.) Bertol.
redstem	*Erodium cicutarium* (L.) L'Hér.
fireweed	*Epilobium angustifolium* L.
fleabane	
annual	*Erigeron annuus* (L.) Pers.
Canada (*see* horseweed)	
dwarf	*Erigeron divaricatus* Michx.
rough	*Erigeron strigosus* Muhl.
flixweed	*Descurainia sophia* (L.) Webb.
see also tansymustard	
flower-of-an-hour (*see* Venice mallow)	
fogfruit, wedgeleaf	*Lippia cuneifolia* Steud.
four-o'clock, wild	*Mirabilis nyctaginea* (Michx.) MacM.
foxtail	
giant	*Setaria faberi* Herrm.
green	*Setaria viridis* (L.) Beauv.
water	*Alopecurus geniculatus* L.
yellow	*Setaria glauca* (L.) Beauv.
see also bristlegrass	
franseria	
bur	*Franseria discolor* Nutt.
woollyleaf	*Franseria tomentosa* Gray
galinsoga	
hairy	*Galinsoga ciliata* (Raf.) Blake
smallflower	*Galinsoga parviflora* Cav.
garlic, wild	*Allium vineale* L.
see also onion	

Common Name	Scientific Name
gaura	
hairy	*Gaura villosa* Torr.
scarlet	*Gaura coccinea* Pursh
scented	*Gaura odorata* Lag.
wavyleaf	*Gaura sinuata* Nutt.
geranium	
Carolina	*Geranium carolinianum* L.
dovefoot	*Geranium molle* L.
goatgrass	
barb	*Aegilops triuncialis* L.
jointed	*Aegilops cylindrica* Host.
goatsbeard (*see* meadow salsify)	
goatsrue	*Galega officinalis* L.
goatweed (*see* Lindheimer croton)	
goldenpea, prairie	*Thermopsis rhombifolia* (Nutt.) Richards.
goldenrod	
Canada	*Solidago canadensis* L.
gray	*Solidago nemoralis* Ait.
Missouri	*Solidago missouriensis* Nutt.
narrowleaf	*Solidago graminifolia* (L.) Salisb. var. *nuttallii* (Greene) Fern.
rigid	*Solidago rigida* L.
rough	*Solidago rugosa* Mill.
western	*Solidago occidentalis* Nutt.
goosefoot	
mapleleaf	*Chenopodium hybridum* L.
nettleleaf	*Chenopodium murale* L.
oakleaf	*Chenopodium glaucum* L.
see also lambsquarters, Mexicantea	
goosegrass	*Eleusine indica* (L.) Gaertn.
gourd, buffalo	*Cucurbita foetidissima* H. B. K.
grama	
sixweeks	*Bouteloua barbata* Lag.
sixweeks needle	*Bouteloua aristidoides* (H.B.K.) Griseb.
gromwell	*Lithospermum officinale* L.
corn	*Lithospermum arvense* L.
western	*Lithospermum ruderale* Dougl.
groundcherry	
clammy	*Physalis heterophylla* Nees
longleaf	*Physalis longifolia* Nutt.
purpleflower	*Physalis lobata* Torr.
smooth	*Physalis subglabrata* Mack. & Bush
Wright	*Physalis wrightii* Gray
groundsel, common	*Senecio vulgaris* L.
see also ragwort	
gumweed	*Grindelia squarrosa* (Pursh) Dunal
hairgrass, silver	*Aira caryophyllea* L.
halogeton	*Halogeton glomeratus* (M. Bieb.) C. A. Mey.
hawkbit	
fall	*Leontodon autumnalis* L.
rough	*Leontodon nudicaulis* (L.) Banks
hawksbeard	
narrowleaf	*Crepis tectorum* L.
smooth	*Crepis capillaris* (L.) Wallr.
western	*Crepis occidentalis* Nutt.
hawkweed	
mouseear	*Hieracium pilosella* L.
orange	*Hieracium aurantiacum* L.
yellow	*Hieracium pratense* Tausch
healall	*Prunella vulgaris* L.

Common Name	Scientific Name
heliotrope	
Indian	*Heliotropium indicum* L.
seaside	*Heliotropium curassavicum* L.
hellebore	
western false	*Veratrum californicum* Durand
white	*Veratrum viride* Ait.
hemlock, poison	*Conium maculatum* L.
hemp	*Cannabis sativa* L.
Indian	*Apocynum cannabinum* L.
hempnettle	*Galeopsis tetrahit* L.
henbane, black	*Hyoscyamus niger* L.
henbit	*Lamium amplexicaule* L.
hogpotato	*Hoffmanseggia densiflora* Benth.
horehound	
water	*Lycopus americanus* Muhl.
white	*Marrubium vulgare* L.
horsenettle, Carolina	*Solanum carolinense* L.
horsetail, field	*Equisetum arvense* L.
horseweed	*Erigeron canadensis* L.
houndstongue	*Cynoglossum officinale* L.
Indian tobacco	*Lobelia inflata* L.
indigo, wild	*Baptisia tinctoria* (L.) R. Br.
iris, Rocky Mountain	*Iris missouriensis* Nutt.
ironweed	
tall	*Vernonia altissima* Nutt.
western	*Vernonia baldwinii* Torr.
ivy, ground	*Nepeta hederacea* (L.) Trevisan
jewelweed	*Impatiens* spp.
jimsonweed	*Datura stramonium* L.
Joepyeweed	*Eupatorium maculatum* L.
Johnsongrass	*Sorgum halepense* (L.) Pers.
jointvetch, northern	*Aeschynomene virginica* (L.) B. S. P.
knapweed	
black	*Centaurea nigra* L.
brown	*Centaurea jacea* L.
diffuse	*Centaurea diffusa* Lam.
Russian	*Centaurea repens* L.
spotted	*Centaurea maculosa* Lam.
squarrose	*Centaurea squarrosa* Roth
see also starthistle	
knawel	*Scleranthus annuus* L.
knotgrass	*Paspalum distichum* L.
knotweed	
erect	*Polygonum erectum* L.
prostrate	*Polygonum aviculare* L.
silversheath	*Polygonum argyrocoleon* Steud.
see also smartweed, ladysthumb	
kochia	*Kochia scoparia* (L.) Schrad.
kudzu	*Pueraria lobata* (Willd.) Ohwi
ladysthumb	*Polygonum persicaria* L.
lambsquarters	
common	*Chenopodium album* L.
narrowleaf	*Chenopodium leptophyllum* Nutt.
see also goosefoot, Mexican tea	
larkspur	
dwarf	*Delphinium tricorne* Michx.
little	*Delphinium bicolor* Nutt.

Common Name	Scientific Name
low	*Delphinium nelsonii* Greene
plains	*Delphinium geyeri* Greene
tall	*Delphinium barbeyi* Huth
tall mountain	*Delphinium scopulorum* Gray
lettuce	
blue	*Lactuca pulchella* (Pursh) DC.
prickly	*Lactuca scariola* L.
tall	*Lactuca canadensis* L.
licorice, wild	*Glycyrrhiza lepidota* (Nutt.) Pursh
lobelia, great	*Lobelia siphilitica* L.
loco	
bigbend	*Astragalus earlei* Greene
blue	*Astragalus diphysus* Gray
spotted	*Astragalus lentiginosus* Dougl.
woolly	*Astragalus mollissimus* Torr.
white	*Oxytropis lambertii* Pursh
see also milkvetch	
loosestrife, purple	*Lythrum salicaria* L.
lovegrass, tufted	*Eragrostis pectinacea* (Michx.) Nees
lupine	
perennial	*Lupinus perennis* L.
silky	*Lupinus sericeus* Pursh
silvery	*Lupinus argenteus* Pursh
tailcup	*Lupinus caudatus* Kell.
velvet	*Lupinus leucophyllus* Dougl.
madder, field	*Sherardia arvensis* L.
mallow	
cheese (*see* common mallow)	
common	*Malva neglecta* Wallr.
dwarf	*Malva rotundifolia* L.
little	*Malva parviflora* L.
musk	*Malva moschata* L.
Venice	*Hibiscus trionum* L.
marestail (*see* horseweed)	
marshelder	*Iva xanthifolia* Nutt.
mayweed	*Anthemis cotula* L.
medic, black	*Medicago lupulina* L.
Mediterraneangrass	*Schismus barbatus* (L.) Thell.
medusahead	*Elymus caput-medusae* L.
mercury, threeseed	*Acalypha virginica* L.
Mexicantea	*Chenopodium ambrosioides* L.
milkvetch	
narrowleaf	*Astragalus pectinatus* Hook.
two grooved	*Astragalus bisulcatus* Hook.
see also loco	
milkweed	
broadleaf	*Asclepias latifolia* (Torr.) Raf.
butterfly	*Asclepias tuberosa* L.
climbing	*Ampelamus albidus* (Nutt.) Britt.
common	*Asclepias syriaca* L.
dwarf	*Asclepias ovalifolia* Dcne
eastern whorled	*Asclepias subverticillata* (Gray) Vail
labriform	*Asclepias labriformis* Jones
Mexican	*Asclepias mexicana* Cav.
Mexican whorled	*Asclepias fascicularis* Decne.
showy	*Asclepias speciosa* Torr.
western whorled	*Asclepias verticillata* L.
woollypod	*Asclepias eriocarpa* Benth.
mint	
field	*Mentha arvensis* L.

Common Name	Scientific Name
horse	*Monarda* spp.
moneywort	*Lysimachia nummularia* L.
monolepis	*Monolepis nuttallianus* (Schult.) Greene
morningglory	
bigroot	*Ipomoea pandurata* (L.) G. F. W. Mey
common	*Ipomoea purpurea* (L.) Roth
ivyleaf	*Ipomoea hederacea* (L.) Jacq.
scarlet	*Ipomoea coccinea* L.
woolly	*Ipomoea hirsutula* Jacq. f.
motherwort	*Leonurus cardiaca* L.
mugwort	*Artemisia vulgaris* L.
muhly	
Mexican	*Muhlenbergia mexicana* (L.) Trin.
wirestem	*Muhlenbergia frondosa* (Poir.) Fern.
see also nimblewill	
mulesears	*Wyethia amplexicaulis* Nutt.
mullein	
common	*Verbascum thapsus* L.
moth	*Verbascum blattaria* L.
turkey	*Eremocarpus setigerus* Benth.
mustard	
ball	*Neslia paniculata* (L.) Desv.
black	*Brassica nigra* (L.) Koch.
blue	*Chorispora tenella* DC.
dog	*Erucastrum gallicum* (Willd.) E. O. Schulz
hairy	*Hirschfeldia incana* (L.) Lagreze-Fosset
haresear	*Conringia orientalis* (L.) Dum.
hedge	*Sisymbrium officinale* (L.) Scop.
Indian	*Brassica juncea* (L.) Coss.
Jim Hill (*see* tumble mustard)	
tall hedge	*Sisymbrium loeselii* L.
tansy-	*Descurainia pinnata* (Walt.) Britt.
tumble	*Sisymbrium altissimum* L.
white	*Brassica hirta* Moench
wild	*Brassica kaber* (DC.) L. C. Wheeler var. *pinnatifida* (Stokes) L. C. Wheeler
wormseed	*Erysimum cheiranthoides* L.
see also London rocket	
needleandthread	*Stipa comata* Trin. & Rupr.
nettle	
bull	*Cnidoscolus stimulosus* (Michx.) Gray
burning	*Urtica urens* L.
Carolina horse-	*Solanum carolinense* L.
hedge	*Stachys arvensis* L.
hemp-	*Galeopsis tetrahit* L.
stinging	*Urtica dioica* L.
tall	*Urtica procera* Muhl.
nightshade	
bitter	*Solanum dulcamara* L.
black	*Solanum nigrum* L.
cutleaf	*Solanum triflorum* Nutt.
hairy	*Solanum villosum* Mill.
silverleaf	*Solanum elaeagnifolium* Cav.
see also horsenettle	
nimblewill	*Muhlenbergia schreberi* J. F. Gmel.
see also muhly	
nutgrass (*see* nutsedge)	
nutsedge	
purple	*Cyperus rotundus* L.
yellow	*Cyperus esculentus* L.

Common Name	Scientific Name
oat	
slender	*Avena barbata* Brot.
wild	*Avena fatua* L.
oatgrass	
poverty	*Danthonia spicata* (L.) Beauv.
tall	*Arrhenatherum elatius* (L.) Presl
onion, wild	*Allium canadense* L.
see also garlic	
orache	
halberdleaf	*Atriplex patula* L. var. *hastata* (L.) Gray
red	*Atriplex rosea* L.
spreading	*Atriplex patula* L.
orchardgrass	*Dactylis glomeratus* L.
panicum, fall	*Panicum dichotomiflorum* Michx.
parsnip	
water	*Sium suave* Walt.
wild	*Pastinaca sativa* L.
partridgepea	*Cassia fasciculata* Michx.
paspalum, fringeleaf	*Paspalum ciliatifolium* Michx.
passionflower, Maypop	*Passiflora incarnata* L.
peaweed, Austrian	*Swainsona salsula* (Pall.) Taub.
pennycress	
field	*Thlaspi arvense* L.
thoroughwort	*Thlaspi perfoliatum* L.
pennywort, lawn	*Hydrocotyle sibthorpioides* Lam.
pepperweed	
clasping	*Lepidium perfoliatum* L.
field	*Lepidium campestre* (L.) R. Br.
greenflower	*Lepidium densiflorum* Schrad.
perennial	*Lepidium latifolium* L.
Virginia	*Lepidium virginicum* L.
periwinkle	*Vinca minor* L.
pigweed	
prostrate	*Amaranthus graecizans* L.
redroot	*Amaranthus retroflexus* L.
rough (*see* redroot pigweed)	
Russian	*Axyris amaranthoides* L.
smooth	*Amaranthus hybridus* L.
spiny	*Amaranthus spinosus* L.
tumble	*Amaranthus albus* L.
winged	*Cycloloma atriplicifolium* (Spreng.) Coult.
see also amaranth	
pineappleweed	*Matricaria matricarioides* (Less.) Porter
pimpernel, scarlet	*Anagallis arvensis* L.
plantain	
blackseed	*Plantago rugelii* Dcne.
bracted	*Plantago aristata* Michx.
broadleaf	*Plantago major* L.
buckhorn	*Plantago lanceolata* L.
slender	*Plantago pusilla* Nutt.
woolly	*Plantago purshii* Roem. & Schult.
pokeweed	*Phytolacca americana* L.
poorjoe	*Diodia teres* Walt.
poppy, Roemer	*Roemeria refracta* DC.
porcupinegrass	*Stipa spartea* Trin.
potato, hog	*Hoffmanseggia densiflora* Benth.
pricklepoppy	*Argemone intermedia* Sweet
Mexican	*Argemone mexicana* L.
povertyweed	*Iva axillaris* Pursh
prairietea	*Croton monanthogynus* Michx.

Common Name	Scientific Name
primrose, evening	
(*see* eveningprimrose)	
puccoon (*see* gromwell)	
puncturevine	*Tribulus terrestris* L.
purslane	
common	*Portulaca oleracea* L.
horse	*Trianthema portulacastrum* L.
quackgrass	*Agropyron repens* (L.) Beauv.
queensdelight	*Stillingia sylvatica* L.
rabbitfootgrass	*Polypogon monspeliensis* (L.) Desf.
radish, wild	*Raphanus raphanistrum* L.
ragweed	
blood	*Ambrosia trifida* L. var. *texana* Scheele
common	*Ambrosia artemisiifolia* L.
giant	*Ambrosia trifida* L.
lanceleaf	*Ambrosia bidentata* Michx.
perennial	*Ambrosia psilostachya* DC.
western (*see* perennial ragweed)	
see also franseria	
ragwort	
golden	*Senecio aureus* L.
tansy	*Senecio jacobaea* L.
see also groundsel	
rattlebox	*Crotalaria sagittalis* L.
see also crotalaria	
redtop	*Agrostis alba* L.
reed, common	*Phragmites communis* Trin.
reed canarygrass	*Phalaris arundinacea* L.
rescuegrass	*Bromus catharticus* Vahl
rice	
jungle-	*Echinochloa colonum* (L.) Link
red	*Oryza sativa* L.
rocket	
London	*Sisymbrium irio* L.
yellow	*Barbarea vulgaris* R. Br.
rubberweed	
bitter	*Hymenoxys odorata* DC.
Colorado	*Hymenoxys richardsoni* (Hook.) Cockerell
rush	
slender	*Juncus tenuis* Willd.
spike	*Eleocharis* sp.
toad	*Juncus bufonius* L.
ryegrass, Italian	*Lolium multiflorum* Lam.
sage	
Mediterranean	*Salvia aethiopis* L.
prairie	*Artemisia ludoviciana* Nutt. var. *gnaphalodes* (Nutt.) Torr. & Gray
salsify	
common	*Tragopogon porrifolius* L.
meadow	*Tragopogon pratensis* L.
western	*Tragopogon major* Jacq.
saltbush	*Atriplex argentea* Nutt.
wheelscale	*Atriplex elegans* (Moq.) D. Dietr.
Wright	*Atriplex wrightii* Wats.
see also orache	
sandbur	
field	*Cenchrus pauciflorus* Benth.
longspine	*Cenchrus longispinus* (Hack.) Fern.
southern	*Cenchrus echinatus* L.

Common Name	Scientific Name
sandwort, thymeleaf	*Arenaria serpyllifolia* L.
sesbania	*Sesbania exaltata* (Raf.) Cory
shepherdspurse	*Capsella bursa-pastoris* (L.) Medic.
sicklepod	*Cassia tora* L.
sida	
alkali	*Sida hederacea* (Dougl.) Torr.
prickly	*Sida spinosa* L.
silverweed	*Potentilla anserina* L.
skeletonweed	*Lygodesmia juncea* (Pursh) D. Don.
smartweed	
green	*Polygonum scabrum* Moench.
hedge	*Polygonum scandens* L.
mild	*Polygonum hydropiperoides* Michx.
pale	*Polygonum lapathifolium* L.
Pennsylvania	*Polygonum pensylvanicum* L.
swamp	*Polygonum coccineum* Muhl.
see also knotweed, ladysthumb	
smutgrass	*Sporobolus poiretii* (Roem. & Schult.) Hitchc.
snakeroot	
button	*Eryngium yuccifolium* Michx.
white	*Eupatorium rugosum* Houtt.
snakeweed	
broom	*Gutierrezia sarothrae* (Pursh) Britt. & Rusby
threadleaf	*Gutierrezia microcephala* (DC.) Gray
see also broomweed	
sneezeweed	
bitter	*Helenium tenuifolium* Nutt.
common	*Helenium autumnale* L.
western	*Helenium hoopesii* Gray
snow-on-the-mountain	*Euphorbia marginata* Pursh
sorrel	*Rumex acetosa* L.
red	*Rumex acetosella* L.
sheep (*see* red sorrel)	
sowthistle	
annual	*Sonchus oleraceus* L.
perennial	*Sonchus arvensis* L.
spiny	*Sonchus asper* (L.) Hill.
Spanishneedles	*Bidens bipinnata* L.
see also beggarticks	
spearmint	*Mentha spicata* L.
speedwell	
common	*Veronica officinalis* L.
corn	*Veronica arvensis* L.
creeping	*Veronica filiformis* Sm.
purslane	*Veronica peregrina* L.
thymeleaf	*Veronica serpyllifolia* L.
sprangletop	
Mexican	*Leptochloa uninervia* (Presl.) Hitchc. & Chase
red	*Leptochloa filiformis* (Lam.) Beauv.
spurge	
cypress	*Euphorbia cyparissias* L.
flowering	*Euphorbia corollata* L.
hairy	*Euphorbia vermiculata* Raf.
leafy	*Euphorbia esula* L.
petty	*Euphorbia peplus* L.
prostrate	*Euphorbia supina* Raf.
shining	*Euphorbia lucida* Waldst. & Kit
spotted	*Euphorbia maculata* L.
sun	*Euphorbia helioscopia* L.
see also snow-on-the-mountain	
spurry, corn	*Spergula arvensis* L.

Common Name	Scientific Name
squirreltail	*Sitanion hystrix* (Nutt.) J. G. Smith
starthistle	
Iberian	*Centaurea iberica* Trevir.
Malta	*Centaurea melitensis* L.
purple	*Centaurea calcitrapa* L.
yellow	*Centaurea solstitialis* L.
see also knapweed	
sticktight	
European	*Lappula echinata* Gilib.
western	*Lappula occidentalis* (S. Wats.) Greene
stickseed (*see* sticktight)	
stinkgrass	*Eragrostis cilianensis* (All.) Vignolo Lutati
stitchwort	*Stellaria graminea* L.
St. Johnswort	*Hypericum perforatum* L.
spotted	*Hypericum punctatum* L.
sumpweed, rough	*Iva ciliata* Willd.
bigleaf (*see* marshelder)	
sunflower	*Helianthus annuus* L.
California	*Helianthus californicus* DC.
prairie	*Helianthus petiolaris* Nutt.
sweetgrass	*Hierochloe odorata* (L.) Beauv.
swinecress	*Coronopus didymus* (L.) Smith
switchgrass	*Panicum virgatum* L.
tansy	*Tanacetum vulgare* L.
tansymustard	*Descurainia pinnata* (Walt.) Britt.
see also flixweed	
tarweed	
Chilean	*Madia sativa* Mol.
cluster	*Madia glomerata* Hook.
common	*Hemizonia congesta* DC.
showy	*Madia elegans* D. Don
teasel	*Dipsacus sylvestris* Huds.
telegraphplant	*Heterotheca grandiflora* Nutt.
thistle	
blessed	*Cnicus benedictus* L.
blue	*Echium vulgare* L.
bull	*Cirsium vulgare* (Savi) Tenore
Canada	*Cirsium arvense* (L.) Scop.
Flodman	*Cirsium flodmani* (Rydb.) Arthur
Italian	*Carduus pycnocephalus* L.
milk	*Silybum marianum* (L.) Gaertn.
musk	*Carduus nutans* L.
pasture	*Cirsium pumilum* Spreng.
plumeless	*Carduus acanthoides* L.
Russian	*Salsola kali* L. var. *tenuifolia* Tausch
tall	*Cirsium altissimum* (L.) Spreng.
threeawn	
prairie	*Aristida oligantha* Michx.
red	*Aristida longiseta* Steud.
thyme, creeping	*Thymus serpyllum* L.
tickseed	*Coreopsis tinctoria* Nutt.
timothy	*Phleum pratense* L.
toadflax	
Dalmatian	*Linaria dalmatica* Mill.
yellow	*Linaria vulgaris* Hill
tobacco, Indian	*Lobelia inflata* L.
tumblegrass	*Schedonnardus paniculatus* (Nutt.) Trel.
tumbleweed (*see* tumble pigweed)	
turkey mullein	*Eremocarpus setigerus* Benth.
turnip, wild	*Brassica campestris* L.

Common Name	Scientific Name
velvetgrass	*Holcus lanatus* L.
German	*Holcus mollis* L.
velvetleaf	*Abutilon theophrasti* Medic.
Venus lookingglass	*Specularia perfoliata* (L.) A. DC.
verbena (*see* vervain)	
vernalgrass, sweet	*Anthoxanthum odoratum* L.
vervain	
blue	*Verbena hastata* L.
hoary	*Verbena stricta* Vent.
prostrate	*Verbena bracteata* Lag. & Rodr.
white	*Verbena urticifolia* L.
vetch	
common	*Vicia sativa* L.
narrowleaf	*Vicia angustifolia* L.
wallflower, western	*Erysimum asperum* (Nutt.) DC.
waterhemlock	
spotted	*Cicuta maculata* L.
western	*Cicuta douglasii* (DC.) Coult. & Rose
water parsnip	*Sium suave* Walt.
watergrass (*see* barnyardgrass)	
wheatgrass, western	*Agropyron smithii* Rydb.
whitetop (*see* hoary cress)	
hairy	*Cardaria pubescens* (C. A. Meyer) Rollins
whitlowwort	*Draba verna* L.
willowweed	
hairy	*Epilobium hirsutum* L.
panicle	*Epilobium paniculatum* Nutt.
windmillgrass, tumble	*Chloris verticillata* Nutt.
wintercress	
bitter (*see* yellow rocket)	
early	*Barbarea verna* (Mill.) Aschers.
witchgrass	*Panicum capillare* L.
witchweed	*Striga asiatica* (L.) Kuntze
woodsorrel	
European	*Oxalis europaea* Jord.
yellow	*Oxalis stricta* L.
wormwood	
absinth	*Artemisia absinthium* L.
annual	*Artemisia annua* L.
biennial	*Artemisia biennis* Willd.
Louisiana	*Artemisia ludoviciana* Nutt.
woundwort, marsh	*Stachys palustris* L.
yankeeweed	*Eupatorium compositifolium* Walt.
yarrow	*Achillea millefolium* L.
western	*Achillea lanulosa* Nutt.

ANNUAL AND PERENNIAL HERBACEOUS WEEDS
(arranged alphabetically by scientific name)

Scientific Name	Common Name
Abutilon theophrasti	velvetleaf
Acalypha virginica	threeseed mercury
Achillea lanulosa	western yarrow
A. millefolium	common yarrow
Actaea rubra	red baneberry
Aegilops cylindrica	jointed goatgrass
A. triuncialis	barb goatgrass
Aeschynomene virginica	northern jointvetch
Agrimonia gryposepala	agrimony
Agropyron repens	quackgrass
A. smithii	western wheatgrass
Agrostemma githago	corn cockle
Agrostis alba	redtop
Aira caryophyllea	silver hairgrass
Allium canadense	wild onion
A. vineale	wild garlic
Alopecurus geniculatus	water foxtail
Amaranthus albus	tumble pigweed
A. graecizans	prostrate pigweed
A. hybridus	green amaranth
A. palmeri	Palmer amaranth
A. retroflexus	rough pigweed
A. spinosus	spiny amaranth
Ambrosia artemisiifolia	common ragweed
A. bidentata	lanceleaf ragweed
A. psilostachya	perennial ragweed
A. trifida	giant ragweed
A. trifida var. *texana*	blood ragweed
Ampelamus albidus	climbing milkweed
Amsinckia douglasiana	Douglas fiddleneck
A. intermedia	coast fiddleneck
A. tessellata	western fiddleneck
Anagallis arvensis	scarlet pimpernel
Anaphalis margaritacea	pearly everlasting
Andropogon glomeratus	bushy beardgrass
A. scoparius	little bluestem
A. virginicus	broomsedge
Antennaria plantaginifolia	plantainleaf everlasting
Anthemis arvensis	corn chamomile
A. cotula	mayweed
A. tinctoria	yellow chamomile
Anthoxanthum odoratum	sweet vernalgrass
Apocynum androsaemifolium	spreading dogbane
A. cannabinum	Indian hemp
Arctium lappa	great burdock
A. minus	common burdock
Arenaria serpyllifolia	thymeleaf sandwort
Argemone intermedia	pricklepoppy
A. mexicana	Mexican pricklepoppy
Aristida longiseta	red threeawn
A. oligantha	prairie threeawn
Arrhenatherum elatius	tall oatgrass
Artemisia absinthium	absinth wormwood
A. annua	annual wormwood
A. biennis	biennial wormwood
A. ludoviciana	Louisiana wormwood
A. ludoviciana var. *gnaphalodes*	prairie sage
A. vulgaris	mugwort

Scientific Name	Common Name
Asclepias eriocarpa	woollypod milkweed
A. fascicularis	Mexican whorled milkweed
A. incarnata	swamp milkweed
A. labriformis	labriform milkweed
A. latifolia	broadleaf milkweed
A. mexicana	Mexican milkweed
A. ovalifolia	dwarf milkweed
A. speciosa	showy milkweed
A. subverticillata	western whorled milkweed
A. syriaca	common milkweed
A. tuberosa	butterfly milkweed
A. verticillata	eastern whorled milkweed
Aster exilis	slender aster
A. parryi	woody aster
A. pilosus	white heath aster
A. simplex	white field aster
A. spinosus	spiny aster
Astragalus bisulcatus	two-grooved milkvetch
A. diphysus	blue loco
A. earlei	bigbend loco
A. lentiginosus	spotted loco
A. mollissimus	woolly loco
A. pectinatus	narrowleaf milkvetch
Atriplex argentea	saltbush
A. elegans	wheelscale saltbush
A. patula	spreading orache
A. patula var. *hastata*	halberdleaf orache
A. rosea	red orache
A. wrightii	Wright saltbush
Avena barbata	slender oat
A. fatua	wild oat
Axyris amaranthoides	Russian pigweed
Baptisia tinctoria	wild indigo
Barbarea verna	early wintercress
B. vulgaris	yellow rocket
Bassia hyssopifolia	fivehook bassia
Bellis perennis	English daisy
Berteroa incana	hoary alyssum
Bidens bipinnata	Spanishneedles
B. frondosa	devils beggarticks
B. vulgata	tall beggarticks
Bouteloua aristidoides	sixweeks needle grama
B. barbata	sixweeks grama
Brassica campestris	wild turnip
B. hirta	white mustard
B. juncea	Indian mustard
B. kaber var. *pinnatifida*	wild mustard
B. nigra	black mustard
Bromus catharticus	rescuegrass
B. commutatus	hairy chess
B. japonicus	Japanese brome
B. mollis	soft cheess
B. racemosus	upright brome
B. rigidus	ripgut brome
B. rubens	red brome
B. secalinus	cheat
B. tectorum	downy brome
Camelina microcarpa	smallseed falseflax
C. sativa	largeseed falseflax

Scientific Name	Common Name
Campanula rapunculoides	creeping bellflower
Cannabis sativa	hemp
Capsella bursa-pastoris	shepherdspurse
Cardamine spp.	bittercress
Cardaria draba	hoary cress
C. pubescens	hairy whitetop
Carduus acanthoides	plumeless thistle
C. nutans	musk thistle
C. pycnocephalus	Italian thistle
Cassia fasciculata	partridgepea
C. tora	sicklepod
Castilleja spp.	paintbrush
Cenchrus echinatus	southern sandbur
C. longispinus	longspine sandbur
C. pauciflorus	field sandbur
Centaurea calcitrapa	purple starthistle
C. cyanus	cornflower
C. diffusa	diffuse knapweed
C. iberica	Iberian starthistle
C. jacea	brown knapweed
C. maculosa	spotted knapweed
C. melitensis	Malta starthistle
C. nigra	black knapweed
C. repens	Russian knapweed
C. solstitialis	yellow starthistle
C. squarrosa	squarrose knapweed
Cerastium arvense	field chickweed
C. vulgatum	mouseear chickweed
Chenopodium album	common lambsquarters
C. ambrosioides	Mexicantea
C. glaucum	oakleaf goosefoot
C. hybridum	mapleleaf goosefoot
C. leptophyllum	narrowleaf lambsquarters
C. murale	nettleleaf goosefoot
Chloris verticillata	tumble windmillgrass
Chorispora tenella	blue mustard
Chrysanthemum leucanthemum	oxeye daisy
Cichorium intybus	chicory
Cicuta douglasii	western waterhemlock
C. maculata	spotted waterhemlock
Cirsium altissimum	tall thistle
C. arvense	Canada thistle
C. flodmani	Flodman thistle
C. pumilum	pasture thistle
C. vulgare	bull thistle
Cleome serrulata	Rocky Mountain beeplant
Cnicus benedictus	blessed thistle
Cnidoscolus stimulosus	bull nettle
Commelina communis	dayflower
Conium maculatum	poison hemlock
Conringia orientalis	haresear mustard
Convolvulus arvensis	field bindweed
C. sepium	hedge bindweed
Coreopsis tinctoria	tickseed
Coronopus didymus	swinecress
Crepis capillaris	smooth hawksbeard
C. occidentalis	western hawksbeard
C. tectorum	narrowleaf hawksbeard
Crotalaria sagittalis	rattlebox
C. spectabilis	showy crotalaria
Croton capitatus	woolly croton

Scientific Name	Common Name
C. lindheimeri	Lindheimer croton
C. monanthogynus	prairietea
C. texensis	Texas croton
Cucurbita foetidissima	buffalo gourd
Cuphea petiolata	clammy cuphea
Cuscuta campestris	field dodder
C. epilinum	flax dodder
C. epithymum	clover dodder
C. gronovii	swamp dodder
C. indecora	largeseed dodder
C. planiflora	smallseed dodder
C. racemosa var. *chilcana*	Chilean dodder
Cycloloma atriplicifolium	winged pigweed
Cynodon dactylon	Bermudagrass
Cynoglossum officinale	houndstongue
Cyperus esculentus	yellow nutsedge
C. rotundus	purple nutsedge
Dactylis glomerata	orchardgrass
Danthonia spicata	poverty oatgrass
Datura stramonium	jimsonweed
Daubentonia texana	coffeeweed
Daucus carota	wild carrot
Delphinium barbeyi	tall larkspur
D. bicolor	little larkspur
D. geyeri	plains larkspur
D. nelsonii	low larkspur
D. scopulorum	tall mountain larkspur
D. tricorne	dwarf larkspur
Descurainia pinnata	tansymustard
D. sophia	flixweed
Desmodium tortuosum	Florida beggarweed
Digitaria filiformis	slender crabgrass
D. ischaemum	smooth crabgrass
D. sanguinalis	large crabgrass
Diodia teres	poorjoe
Dipsacus sylvestris	teasel
Draba verna	whitlowwort
Echinochloa colonum	junglerice
E. crus-galli	barnyardgrass
Echinocystis lobata	wild cucumber
E. oregona	western wild cucumber
Echium vulgare	blue thistle
Eleocharis spp.	spike rush
Eleusine indica	goosegrass
Elymus caput-medusae	medusahead
Epilobium angustifolium	fireweed
E. hirsutum	hairy willowweed
E. paniculatum	panicle willowweed
Equisetum arvense	field horsetail
Eragrostis cilianensis	stinkgrass
E. pectinacea	tufted lovegrass
Eremocarpus setigerus	turkey mullein
Erigeron annuus	annual fleabane
E. canadensis	horseweed
E. divaricatus	dwarf fleabane
E. strigosus	rough fleabane
Erodium botrys	broadleaf filaree
E. cicutarium	redstem filaree
Erucastrum gallicum	dog mustard

Scientific Name	Common Name
Eryngium hookeri	Hooker eryngo
E. yuccifolium	button snakeroot
Erysimum asperum	western wallflower
E. cheiranthoides	wormseed mustard
Eupatorium capillifolium	dog fennel
E. compositifolium	yankeeweed
E. maculatum	Joepyeweed
E. perfoliatum	boneset
E. rugosum	white snakeroot
Euphorbia corollata	flowering spurge
E. cyparissias	cypress spurge
E. esula	leafy spurge
E. helioscopia	sun spurge
E. lucida	shining spurge
E. maculata	spotted spurge
E. marginata	snow-on-the-mountain
E. peplus	petty spurge
E. supina	prostrate spurge
E. vermiculata	hairy spurge
Fagopyrum tataricum	Tartary buckwheat
Festuca megalura	foxtail fescue
F. myuros	rattail fescue
F. octoflora	sixweeks fescue
Foeniculum vulgare	common fennel
Franseria discolor	bur franseria
F. tomentosa	woollyleaf franseria
Galega officinalis	goatsrue
Galeopsis tetrahit	hempnettle
Galinsoga ciliata	hairy galinsoga
G. parviflora	smallflower galinsoga
Galium aparine	bedstraw
G. asprellum	rough bedstraw
G. boreale	northern bedstraw
G. mollugo	smooth bedstraw
G. verum	yellow bedstraw
Gaura coccinea	scarlet gaura
G. odorata	scented gaura
G. sinuata	wavyleaf gaura
G. villosa	hairy gaura
Geranium carolinianum	Carolina geranium
G. molle	dovefoot geranium
Glechoma hederacea (=*Nepeta hederacea*)	
Glycyrrhiza lepidota	wild licorice
Gnaphalium obtusifolium	fragrant everlasting
G. uliginosum	low cudweed
Grindelia squarrosa	gumweed
Gutierrezia dracunculoides	common broomweed
G. microcephala	threadleaf snakeweed
G. sarothrae	broom snakeweed
G. texana	Texas broomweed
Halogeton glomeratus	halogeton
Hedeoma pulegioides	American pennyroyal
Helenium autumnale	common sneezeweed
H. hoopesii	western sneezeweed
H. tenuifolium	bitter sneezeweed
Helianthus annuus	sunflower
H. californicus	California sunflower
H. ciliaris	Texas blueweed

Scientific Name	Common Name
H. petiolaris	prairie sunflower
H. tuberosus	Jerusalum artichoke
Heliotropium curassavicum	seaside heliotrope
H. indicum	Indian heliotrope
Hemizonia congesta	common tarweed
Heterotheca grandiflora	telegraphplant
H. subaxillaris	camphorweed
Hibiscus trionum	Venice mallow
Hieracium aurantiacum	orange hawkweed
H. pratense	yellow hawkweed
H. pilosella	mouseear hawkweed
Hierochloa odorata	sweetgrass
Hirschfeldia incana	hairy mustard
Hoffmanseggia densiflora	hogpotato
Holcus lanatus	velvetgrass
H. mollis	German velvetgrass
Hordeum brachyantherum	meadow barley
H. jubatum	foxtail barley
H. leporinum	wild barley
H. pusillum	little barley
Hydrocotyle sibthorpioides	lawn pennywort
Hymenoxys odorata	bitter rubberweed
H. richardsoni	Colorado rubberweed
Hyoscyamus niger	black henbane
Hypericum perforatum	St. Johnswort
H. punctatum	spotted St. Johnswort
Hypochoeris radicata	spotted catsear
Impatiens spp.	jewelweed
Ipomoea coccinea	scarlet morningglory
I. hederacea	ivyleaf morningglory
I. hirsutula	woolly morningglory
I. pandurata	bigroot morningglory
I. purpurea	common morningglory
Iris missouriensis	Rocky Mountain iris
Iva axillaris	povertyweed
I. ciliata	rough sumpweed
I. xanthifolia	marshelder
Juncus bufonius	toad rush
J. tenuis	slender rush
Kochia scoparia	kochia
Lactuca canadensis	tall lettuce
L. pulchella	blue lettuce
L. scariola	prickly lettuce
Lamium amplexicaule	henbit
L. maculatum	spotted deadnettle
L. purpureum	red deadnettle
Lappula echinata	European sticktight
L. occidentalis	western sticktight
Leontodon autumnalis	fall hawkbit
L. nudicaulis	rough hawkbit
Leonurus cardiaca	motherwort
Lepidium campestre	field pepperweed
L. densiflorum	greenflower pepperweed
L. latifolium	perennial pepperweed
L. perfoliatum	yellowflower pepperweed
L. virginicum	Virginia pepperweed
Leptochloa filiformis	red sprangletop

Scientific Name	Common Name
L. uninervia	Mexican sprangletop
Linaria dalmatica	Dalmatian toadflax
L. vulgaris	yellow toadflax
Lippia cuneifolia	wedgeleaf fogfruit
Lithospermum arvense	corn gromwell
L. officinale	gromwell
L. ruderale	western gromwell
Lobelia inflata	Indian tobacco
L. siphilitica	great lobelia
Lolium multiflorum	Italian ryegrass
L. temulentum	darnel
Lupinus argenteus	silvery lupine
L. caudatus	tailcup lupine
L. leucophyllus	velvet lupine
L. perennis	perennial lupine
L. sericeus	silky lupine
Lychnis alba	white cockle
L. dioica	red campion
Lycopus americanus	water horehound
L. uniflorus	bugleweed
Lygodesmia juncea	skeletonweed
Lysimachia nummularia	moneywort
Lythrum salicaria	purple loosestrife
Madia elegans	showy tarweed
M. glomerata	cluster tarweed
M. sativa	Chilean tarweed
Malva moschata	musk mallow
M. neglecta	common mallow
M. parviflora	little mallow
M. rotundifolia	dwarf mallow
Marrubium vulgare	white horehound
Matricaria maritima var. *agrestis*	false chamomile
M. matricarioides	pineappleweed
Medicago hispida	California burclover
M. lupulina	black medic
Mentha arvensis	field mint
M. spicata	spearmint
Mirabilis nyctaginea	wild four-o'clock
Mollugo verticillata	carpetweed
Monarda spp.	horsemint
Monolepis nuttalliana	monolepis
Muhlenbergia frondosa	wirestem muhly
M. mexicana	Mexican muhly
M. schreberi	nimblewill
Nepeta cataria	catnip
N. hederacea	ground ivy
Neslia paniculata	ball mustard
Oenothera albicaulis	prairie eveningprimrose
O. biennis	common eveningprimrose
O. laciniata	cutleaf eveningprimrose
O. pallida	pale eveningprimrose
Oryza sativa	red rice
Oxalis europaea	European woodsorrel
O. stricta	yellow woodsorrel
Oxytropis lambertii	white loco
Panicum capillare	witchgrass
P. dichotomiflorum	fall panicum

Scientific Name	Common Name
P. virgatum	switchgrass
Paspalum ciliatifolium	fringeleaf paspalum
P. dilatatum	Dallisgrass
P. distichum	knotgrass
Phalaris canariensis	canarygrass
P. arundinacea	reed canarygrass
Passiflora incarnata	maypop passionflower
Pastinaca sativa	wild parsnip
Phleum pratense	timothy
Phragmites communis	common reed
Physalis heterophylla	clammy groundcherry
P. lobata	purpleflower groundcherry
P. longifolia	longleaf groundcherry
P. subglabrata	smooth groundcherry
P. wrightii	Wright groundcherry
Phytolacca americana	pokeweed
Plantago aristata	bracted plantain
P. lanceolata	buckhorn plantain
P. major	broadleaf plantain
P. purshii	woolly plantain
P. pusilla	slender plantain
P. rugelii	blackseed plantain
Poa annua	annual bluegrass
P. compressa	Canada bluegrass
P. pratensis	Kentucky bluegrass
Polanisia graveolens	clammyweed
P. trachysperma	western clammyweed
Polygonum argyrocoleon	silversheath knotweed
P. aviculare	prostrate knotweed
P. coccineum	swamp smartweed
P. convolvulus	wild buckwheat
P. erectum	erect knotweed
P. hydropiperoides	mild smartweed
P. lapathifolium	pale smartweed
P. pensylvanicum	Pennsylvania smartweed
P. persicaria	ladysthumb
P. scabrum	green smartweed
P. scandens	hedge smartweed
Polypogon monspeliensis	rabbitfootgrass
Portulaca oleracea	common purslane
Potentilla anserina	silverweed
P. argentea	silvery cinquefoil
P. canadensis	common cinquefoil
P. intermedia	downy cinquefoil
P. norvegica	rough cinquefoil
P. recta	sulphur cinquefoil
Prunella vulgaris	healall
Pteridium aquilinum	bracken fern
Pueraria lobata	kudzu
Ranunculus abortivus	smallflower buttercup
R. acris	tall buttercup
R. arvensis	corn buttercup
R. bulbosus	bulbous buttercup
R. repens	creeping buttercup
R. sceleratus	celeryleaf buttercup
Raphanus raphanistrum	wild radish
Ratibida columnaris	prairie coneflower
Roemeria refracta	Roemer poppy
Rorippa austriaca	Austrian fieldcress
R. islandica	marshcress

Scientific Name	Common Name
Rudbeckia laciniata	tall coneflower
R. serotina	blackeyed susan
Rumex acetosa	sorrel
R. acetosella	red sorrel
R. altissimus	pale dock
R. conglomeratus	cluster dock
R. crispus	curly dock
R. mexicanus	Mexican dock
R. obtusifolius	broadleaf dock
R. occidentalis	western dock
R. patientia	patience dock
R. venosus	veiny dock
Salsola kali var. *tenuifolia*	Russian thistle
Saponaria officinalis	bouncingbet
Salvia aethiopis	Mediterranean sage
Saponaria vaccaria	cow cockle
Schedonnardus paniculatus	tumblegrass
Schismus barbatus	Mediterraneangrass
Scleranthus annuus	knawel
Senecio aureus	golden ragwort
S. jacobaea	tansy ragwort
S. vulgaris	common groundsel
Sesbania exaltata	sesbania
Setaria faberi	giant foxtail
S. geniculata	knotroot bristlegrass
S. glauca	yellow foxtail
S. lutescens (= *S. glauca*)	
S. verticillata	bur bristlegrass
S. viridis	green foxtail
Sherardia arvensis	field madder
Sicyos angulatus	burcucumber
Sida hederacea	alkali sida
S. spinosa	prickly sida
Silene antirrhina	sleepy catchfly
S. cucubalus	bladder campion
S. dichotoma	hairy catchfly
S. noctiflora	nightflowering catchfly
Silphium perfoliatum	cupplant
Silybum marianum	milk thistle
Sisymbrium altissimum	tumble mustard
S. irio	London rocket
S. loeselii	tall hedge mustard
S. officinale	hedge mustard
Sitanion hystrix	squirreltail
Sium suave	water parsnip
Solanum carolinense	Carolina horsenettle
S. dulcamara	bitter nightshade
S. elaeagnifolium	silverleaf nightshade
S. nigrum	black nightshade
S. rostratum	buffalobur
S. triflorum	cutleaf nightshade
S. villosum	hairy nightshade
Solidago canadensis	Canada goldenrod
S. graminifolia var. *nuttallii*	narrowleaf goldenrod
S. missouriensis	Missouri goldenrod
S. nemoralis	gray goldenrod
S. occidentalis	western goldenrod
S. rigida	rigid goldenrod
S. rugosa	rough goldenrod
Sonchus arvensis	perennial sowthistle

Scientific Name	Common Name
S. asper	spiny sowthistle
S. oleraceus	annual sowthistle
Sorghum halepense (=*Sorgum halepense*)	
Sorgum halepense	Johnsongrass
Specularia perfoliata	Venus lookingglass
Spergula arvensis	corn spurry
Sporobolus neglectus	annual dropseed
S. poiretii	smutgrass
Stachys arvensis	hedge nettle
S. palustris	marsh woundwort
Stellaria graminea	stitchwort
S. media	common chickweed
Stillingia sylvatica	queensdelight
Stipa comata	needleandthread
S. spartea	porcupinegrass
Striga asiatica	witchweed
Swainsona salsula	Austrian peaweed
Tanacetum vulgare	tansy
Taraxacum officinale	dandelion
Thermopsis rhombifolia	prairie goldenpea
Thlaspi arvense	field pennycress
T. perfoliatum	thoroughwort pennycress
Thymus serpyllum	creeping thyme
Tragopogon major	western salsify
T. porrifolius	common salsify
T. pratensis	meadow salsify
Trianthema portulacastrum	horse purslane
Tribulus terrestris	puncturevine
Triglochin maritima	seaside arrowgrass
T. palustris	marsh arrowgrass
Urtica dioica	stinging nettle
U. procera	tall nettle
U. urens	burning nettle
Veratrum californicum	western false hellebore
V. viride	white hellebore
Verbascum blattaria	moth mullein
V. thapsus	common mullein
Verbena bracteata	prostrate vervain
V. hastata	blue vervain
V. stricta	hoary vervain
V. urticifolia	white vervain
Verbesina encelioides var. *exauriculata*	crownbeard
Vernonia altissima	tall ironweed
V. baldwinii	western ironweed
Veronica arvensis	corn speedwell
V. filiformis	creeping speedwell
V. officinalis	common speedwell
V. peregrina	purslane speedwell
V. serpyllifolia	thymeleaf speedwell
Vicia augustifolia	narrowleaf vetch
V. sativa	common vetch
Vinca minor	periwinkle
Wyethia amplexicaulis	mulesears
Xanthium chinense	oriental cocklebur
X. echinatum	beach cocklebur
X. pensylvanicum	common cocklebur

Scientific Name	Common Name
X. spinosum	spiny cocklebur
Xylorrhiza parryi (= *Aster parryi*)	
Zephyranthes atamasco	Atamosco lily
Zigadenus paniculatus	foothill deathcamas
Z. venenosus	meadow deathcamas
Zygophyllum fabago	Syrian beancaper

News and Notes

Mr. J. L. Williams has been added to the Extension Staff in the Department of Botany and Plant Pathology of Purdue University as weed specialist, starting June 1, 1960. Mr. Williams received B.S. and M.S. degrees from Purdue University and expects to receive the Ph.D. degree from Purdue University in September.

The address of Mr. E. J. Bowles, Sec.-Treas. of the Western Weed Control Conference is 3239 Mayfair Blvd., Fresno, California.

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BIBLIOGRAPHY OF WEED INVESTIGATIONS
for
October, November, December 1959

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Crops Research Division
Weed Investigations Section

This bibliography of current literature on weeds and their control is issued as a service to those engaged in weed research in the United States and other countries. In issuing this bibliography the Crops Research Division, Agricultural Research Service, U.S.D.A. does not assume responsibility for the subject matter cited.

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